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THE *IN VITRO* CULTIVATION OF *NIPPOSTRONGYLUS MURIS* TO THE ADULT STAGE

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The cultivation *in vitro* of nematode parasites of vertebrates should prove to be an important tool which might ultimately be used in the study of many aspects of nematode biology, and in the elucidation of some of the complex factors which are involved in the parasite-host relationship. The reviews by Lapage (1937), Hoeppli, Feng and Chu (1938), Hobson (1948), and von Brand (1952) summarized many of the early studies which were concerned primarily with survival of larval or adult nematodes under various conditions. More recently, with the development of axenic procedures, progress has been made in achieving growth and prolonged survival of several species. Glaser and Stoll (1938a, b) and Stoll (1940) were able to cultivate the free-living stages of *Haemonchus contortus,* and also obtained partial differentiation of the parasitic stages of this organism. The axenic cultivation of the free-living stages of five other strongyles was subsequently reported: *Ancylostoma braziliense* by Lawrence (1948), and *A. caninum, A. duodenale, Nippostrongylus muris,* and *Necator americanus* by Weinstein (1949, 1953, 1954). Using roller tube tissue culture as a tool, Weller (1943) found that *Trichinella spiralis* larvae underwent molting, and some worms showed differentiation comparable to early development in the gut. Extremely long periods of survival have been attained for the larva of *Eustrongylides ignotus* by von Brand and Simpson (1945), some remaining alive for as long as 4 years. Pitts and Ball (1955) have reported slight growth of the newly hatched larvae of *Ascaris lumbricoides.*

The studies on *Neoaplectana glaseri* (Glaser, 1940; Stoll, 1953) and *N. chresima* (Glaser, McCoy and Girth, 1942), nematode parasites of insects, and on the free-living organisms, *Rhabditis pellio* and *R. briggsae* (Dougherty and Calhoun, 1948b; Dougherty, 1950; Dougherty and Keith, 1953) have contributed considerably to the fundamental understanding of the nutritional requirements of nematodes.

The present study reports the axenic development of *Nippostrongylus muris* from the egg to the adult, with primary emphasis on the cultivation of the stages corresponding to the "parasitic" part of the cycle. Although some information will be presented on the development of the free-living stages, a more detailed account of this phase will be given in a separate publication.

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THE BIOLOGY OF *Nippostrongylus muris*

Yokogawa (1920; 1922) described the morphology and life cycle of *N. muris* in considerable detail, and Lucker (1936) demonstrated the first molt, which heretofore had been overlooked. The adult worms reside in the anterior portion of the small intestine of the rat, lying in contact with, or partially embedded in the mucosa. Eggs deposited by the female contain embryos in the early stages of segmentation. Development and hatching of the rhabditiform larva occur after approximately 18 to 24 hours outside the host, under appropriate conditions of moisture and oxygen supply. The first rhabditiform larva grows and molts to the second rhabditiform stage, and after a further period of growth, undergoes a second molt to the filariform, or third stage larva. The exsheathed organism averages 635 microns in length. This cycle from egg to filariform larva generally takes 4 to 5 days. Boardman (1933) found that the larvae would develop to the filariform stage when fed on living bacteria only, which presumably is their normal food source in nature. Weinstein (1949, 1954) subsequently reported the axenic cultivation of these free-living stages in a variety of media.

The parasitic cycle is initiated when the filariform larva penetrates the skin of the rat, which is the normal mode of entry into the host. This third stage larva then migrates to the lungs, the majority arriving about 15 hours after infection. In this site the larva feeds primarily on whole blood; blood cells can be seen in the gut lumen in various stages of digestion. Rapid growth and differentiation occur, culminating in the third molt. Larvae begin this ecdysis at about 35 hours after infection. Our observations indicate that at approximately 45 hours, 20% of the larvae recovered from lung tissue have entered this phase. Following completion of the molt, migration to the small intestine takes place, the majority of the worms having arrived there by 60 hours. Further growth occurs in the intestine, with the fourth and final molt to male and female adult stages occurring from about 90 to 120 hours. These fifth-stage worms mature rapidly so that by the sixth day following infection, small numbers of eggs can be found in the host's feces. The worms in the intestine are often situated with their anterior ends penetrating the mucosa, where they apparently feed on traumatized tissue cells, as well as on blood (Porter, 1935a; Taliaferro and Sarles, 1939). There is some evidence that the worms also ingest intestinal contents, since specimens are frequently encountered containing in their gut lumen various species of flagellates parasitizing the intestinal tract of the rat. The male worms range from 3 to 4 mm in length and the females from 4 to 6 mm (Yokogawa, 1920).

METHODS

Preparation of the larval inoculum. The strain of *N. muris* used in these studies was maintained in Sprague-Dawley rats for many years. To rear the free-living stages, cultures were prepared in Petri dishes by mixing charcoal with feces from heavily infected rats. Unlike many other strongyle larvae, the filariform larvae of *N. muris* normally exsheath while on the surface of the charcoal culture, the sheath forming a crumpled cup around the base of the actively extended organism. Such exsheathed larvae were isolated from charcoal cultures in 0.7% saline employing a Baermann apparatus. By use of a fine-mesh muslin cloth lining a wire sieve, very clean yields of larvae free from particulate material were obtained. The

larvae were washed several times in sterile saline in 40-ml conical tubes; they were settled by gravity or slow centrifugation. The organisms were sterilized externally with a dilute hypochlorite solution, as recommended by Lapage (1935) and Glaser and Stoll (1940). Approximately 5,000 to 10,000 washed larvae were suspended in 20 ml of such a solution, made by adding 0.25 ml "Clorox"[1] to 19.75 ml of 0.7% sodium chloride. The larvae were exposed for approximately 15 minutes; the tube was agitated occasionally to keep them suspended. They were then centrifuged, the supernatant discarded, and the larvae transferred to 20 ml sterile saline in a conical tube. The larvae were washed through a total of 3 to 4 such tubes of saline, the final suspension being used to inoculate culture media. It was found unnecessary to attempt to rid larvae of the residual bacteria retained in their gut since these were effectively disposed of by the high concentrations of antibiotics incorporated into the experimental media.

Cultures were inoculated in one of two ways. A few small drops of a calibrated larval suspension were added directly to the culture tube, or a determined number of organisms was settled out in a small conical tube, and the supernatant was removed. The culture medium was then added to the tube, and the resulting suspension transferred in entirety to the culture vessel. Approximately 175 to 400 larvae were added per culture.

Preparation of 50% chick embryo extract (CEE_{50}). Nine- or ten-day-old embryos were removed aseptically from the egg to a covered beaker embedded in dry ice, so that the embryos were frozen rapidly. They were usually processed immediately or on the following day, being stored at – 20° C during the intervening period. The embryos were homogenized for 60 seconds in a Waring blendor with chilled Tyrode solution adjusted to pH 7.2, in a ratio of 1:1 (weight embryos: volume Tyrode). After standing at 5° C for approximately 1 hour, the homogenate was then centrifuged at 2,000 rpm for 15 minutes at zero degrees. The supernatant fluid was removed, frozen in a dry ice-alcohol bath, and stored at – 20° C until used. This material is referred to as 50% chick embryo extract (CEE_{50}).

Lower concentrations of embryo extract were prepared by diluting CEE_{50} on a volume: volume basis. For example, 100 ml of a medium containing 20 ml CEE_{50} would be considered to contain a final concentration of 10 percent embryo extract.

Antibiotics. A mixture of penicillin and streptomycin was added to all culture media to achieve a final concentration of 300 units penicillin and 0.3 mg streptomycin per ml medium.

Preparation and handling of cultures. All cultures in which the development of the "parasitic" stages were studied were established with filariform larvae derived from charcoal cultures. In most instances cultures were transferred at regular intervals over a period of days or weeks. A sufficient quantity of medium was, therefore, initially mixed sufficient to perform all the scheduled transfers. In media to which embryo extract or serum was added, the mixing vessel was maintained at approximately 5° C in an ice bath. The completed medium was tubed in 2- or 3-ml quantities and frozen immediately in a dry ice-alcohol mixture. The tubes were then stored at – 20° C until needed.

Flask cultures were prepared, unless otherwise stated, in 50-ml Erlenmeyer

[1] "Clorox" (sodium hypochlorite, 5.25% by weight), Clorox Chemical Co., Oakland, California.

flasks which received 3 ml of medium. These were incubated in a stationary position. Tube cultures were prepared in 16 × 150 mm tubes, each of which received 2 ml of medium. The tubes were placed in a horizontal position in a drum which revolved at 12 revolutions per hour. Both flasks and tubes were closed with rubber stoppers. All cultures were incubated at 37.5° C.

Transfers were performed at approximately 24- to 48-hour intervals on most cultures, unless otherwise stated. The culture was taken up in a Pasteur pipette and transferred to a conical tube. The larvae were settled to the bottom of the tube by low speed centrifugation, or by gravity. The supernatant was removed completely, and fresh medium was used to suspend the larvae and transfer them to a new culture flask or tube. Our practice at present, is to keep all tubes in racks which are immersed in shallow pans of water at approximately 37° C during the entire transfer process, so that little cooling occurs.

During the early studies, medium was renewed in tube cultures as follows: Larvae were settled out directly in the culture tubes, the old medium removed, and fresh medium added to the same tube. After a period of days, the walls of such tubes accumulated precipitated material to which the worms tended to adhere, and which also made observation difficult. This procedure was considered unsatisfactory and was not continued.

Examination of cultures. Cultures prepared in flasks were occasionally examined by removing one or two drops, and determining the condition of the larvae that were found. When transfers were performed, the few larvae which were sometimes carried over with the discarded old medium were in many instances also examined.

Tube cultures were examined at regular intervals, usually once every 24 or 48 hours. The tubes were placed in an approximately horizontal position and observed with a dissecting binocular microscope. Only relatively low magnifications, up to 90×, were used under these circumstances. However, considerable morphological detail could be determined in this way. The cultures tended to become turbid, particularly toward the end of a prolonged incubation period, primarily due to the sediment forming from CEE. Even in such tubes, a fair amount of detail could still be observed. It was desirable, however, particularly when adult worms began to appear in cultures, to remove them from the tubes for detailed study, without contaminating or terminating the culture. This was accomplished in the following way. The contents of the culture tube were pipetted into a Petri dish and examined under a binocular dissecting microscope to locate the desired worms. One or more organisms were then removed with a pipette to a small dish of saline. The remaining worms in the Petri dish were then transferred to a conical tube, the dish being flushed with sterile saline to recover all worms. The worms in this tube were sedimented, and finally transferred to a fresh tube of medium, as performed during the regular transfer process, and incubation was continued. Using saline, the isolated worms were washed free of medium and then mounted on slides for observation or photographing. No attempt was made to return worms to a culture, once they had been removed in this way.

Evaluation of cultures. The evaluation of cultures is not yet established on a completely satisfactory basis. The majority of cultures were transferred, and in so doing, particularly when many transfers were performed, worms were constantly being lost in small numbers. In many instances, these were recovered for exami-

nation, but since this could not be done consistently, the cultures were evaluated primarily on the worms present at termination. When only a few transfers were made, the number of worms lost was relatively small. However, in some instances when as many as 20 transfers were performed, only 50 to 75% of the worms were eventually found.

At termination of both flask and tube cultures, the entire contents of the culture vessel were removed and examined in small portions on slides. The stage of development, condition, and other pertinent information were recorded for all worms recovered.

Sterility tests. The components of all media were initially tested for sterility by inoculation into thioglycollate broth. At the termination of an experiment, cultures were similarly inoculated into thioglycollate broth, and occasionally were checked by Gram stain. All cultures, when examined microscopically as fluid mounts, were checked for the presence of bacteria.

EXPERIMENTAL

Sterility tests on the larval suspensions used to inoculate cultures invariably showed the presence of viable bacteria. However, no problem arose with regard to bacterial growth in cultures, due to the high concentrations of antibiotics incorporated in the media. Only one tube, of all the cultures established in the course of these experiments, showed bacterial growth developing, while a single fungus contaminant appeared in another.

Flask Cultures

Previous cultivation studies (Weinstein, 1953, 1954) on the free-living stages of hookworm larvae and *N. muris* demonstrated that high concentrations of chick embryo extract alone would support the growth of the organisms from the egg to the filariform stage (pl. I, Fig. 1). This suggested the possibility that such a medium might stimulate the development of the parasitic stages of *N. muris* if the cultivation temperature were raised to 37.5° C. Numerous such cultures were set up and studied over a period of time, testing different batches of CEE_{50}. The following represents the results of a typical trial. A culture was transferred 5 times during an 8-day period at approximately 32- to 40-hour intervals. The culture was examined on day 21, no further transfers having been performed after day 8. At this time, 99% of the larvae were alive and in excellent condition. Definite development had occurred, comparable to the stages encountered at approximately 24 to 30 hours in the lung of the rat. The larvae showed some increase in size, widening of the gut cells, and the development of the typically deep brown pigmentation of the granules in these cells. The cervical glands had begun to elongate and fill with refractile granular inclusions. No lung molt stages were seen. On occasion, however, in other cultures of this type, a few worms which had completed the third molt and were comparable to early intestinal stages were found; these invariably were dead.

In control cultures in Tyrode solution, the larvae remained active for approximately 3 days, but by that time began to show considerable vacuolization and were all dead by day 4. None ever showed any sign of development.

To determine whether bits of chick embryo tissue would support better develop-

ment than the tissue extract, 2 cultures were established in 125-ml Erlenmeyer flasks, in which an entire minced 10-day-old embryo was overlayed with 10 ml Tyrode in each. After incubation for 6 days, approximately 60% of the larvae were still living but were in poor condition and showed virtually no development. It appeared that the extract was probably more accessible for ingestion than the tissue mince and in this way served as a more satisfactory nutrient.

Since CEE_{50} alone would initiate development of the parasitic stage, many experiments were performed in which it was supplemented in various ways.

A basal medium[2] was prepared which was tested alone, and also with the following substances added respectively (concentrations given in terms of the final medium): 0.3% autolyzed brewer's yeast, 0.3% enzymatic hydrolysate of yeast protein, 0.3% Eli Lilly liver extract 343, 0.3% Eli Lilly liver extract 343 plus 9% rabbit serum, and 0.3% yeast protein hydrolysate plus 9% rabbit serum.

These cultures were handled in approximately the same manner as the CEE_{50} cultures described above, except that they were transferred 6 times at 32- and 40-hour intervals over a period of 10 days, and then examined on days 17 to 19. Little difference developed among these cultures. Most larvae showed differentiation up to a 24-hour lung stage, while a few showed slight wrinkling of the cuticle suggestive of the start of the third molt, but none apparently had occurred.

Since it was possible that the nematodes required a living cell or the by-products of a metabolizing cell in order to complete development, cultures were prepared in which living *Trypanosoma cruzi* were present. This organism was chosen for 2 reasons. It had been shown by Phillips (1950) that *Entamoeba histolytica,* an extremely fastidious parasite, showed good growth when associated with *T. cruzi.* In addition, it was noted on occasion, in examination of *N. muris* adult worms obtained from the gut of a rat, that various species of flagellates normally encountered in the rat intestine were present in great numbers in the gut lumen of some of the nematodes. *T. cruzi* cultures were prepared as described by Phillips and Rees (1950)[3] in the following media: (A) liquid trypticase-serum medium, and (B) fluid trypticase medium overlaying an agar base containing defibrinated rabbit blood and trypticase. These media were inoculated with trypanosomes and incubated for 4 days.

Medium A and the overlay of medium B, both containing a good growth of active trypanosomes, were removed and set up as cultures with *N. muris* larvae. Additional cultures were prepared using these media with trypanosomes, to which CEE was added to achieve a final concentration of 12.5%. As controls on the effect of the trypanosomes, medium A and B lacking *T. cruzi* were set up with and without CEE, as above. Flasks were cotton-stoppered and incubated at 37.5° C. The cultures were evaluated at the end of 4 days.

In the control media not inoculated with trypanosomes, the larvae survived well, and actually showed growth in the cultures to which CEE had been added. Particularly in medium B, approximately 95% of the larvae were living and many

[2] *Basal medium constituents (per ml final medium).* CEE_{∞}, 0.3 ml; p-aminobenzoic acid, folic acid, 0.3 micrograms respectively, calcium pantothenate, pyridoxine HCl, i-inositol, ascorbic acid, 0.6 micrograms respectively, ribose nucleic acid, glutamine, 600 micrograms respectively. All above were dissolved in Tyrode solution.

[3] We wish to thank Mr. Phillips for supplying the *T. cruzi* cultures used in these experiments.

of them were in the late lung stage. However, in comparable cultures containing trypanosomes, the larvae were either all dead as in the case of medium B, or else as in medium A, were 88% alive but showed little or no sign of development. In the former, the trypanosomes had also died, but in the latter most of the flagellates were still alive and active. Larvae were examined from the cultures containing trypanosomes to determine whether any flagellates had been ingested. Although much granular material was noted in the gut lumina indicative of active feeding, no definitely intact trypanosomes were seen. It thus appeared that under the test conditions described, *T. cruzi* had no enhancing effect on the development of *N. muris*.

Roller Tube Cultures

One of the disadvantages inherent in flask culture was the inability to examine the larvae in the culture vessel to note any changes in growth. It was necessary either to terminate the culture or to sample it occasionally, which was not particularly desirable. The roller tube technique developed by Gey and Gey (1936) for the *in vitro* study of vertebrate tissue growth had been used by Weller (1943) in studying *Trichinella spiralis* larvae associated with growing explants of chick embryo tissue. The use of such a rotating tube seemed well suited for cultivation studies with *N. muris*. In the following experiments, all cultures were established in such tubes using fluid media, but no tissue explants or other types of living cells were included.

To determine whether continuous rotation might have an adverse effect on the larvae, cultures were established in roller tubes using CEE_{50} as the medium. Larval development was essentially similar to that which had occurred in flasks. In one instance, transfer was performed only once, midway during a 22-day cultivation period. When terminated, 80% of the larvae were still living, and most were comparable to well-developed lung stages. Other such cultures were subjected to many transfers, done as often as twice daily on each of 14 days, and terminated on day 16. Although the survival rate was well over 90%, the larvae showed no further increase in development. Since the worms exhibited no detrimental effects from rotation, all succeeding cultures were prepared in roller tubes.

In a study of the development of the free-living stages of both *N. muris* and *Necator americanus*, it had been found that standard CEE as well as CEE filtrate (Selas), when supplemented respectively with formalin-killed *Escherichia coli*, showed definite increase in yield of third-stage larvae and in size of the organisms, as compared to control CEE alone (Weinstein, 1954). It appeared that formalin-killed *E. coli* might contain a growth factor for *N. muris* lacking in CEE, or present only in subminimal quantities.

To determine the effect on the development of the parasitic stages, cultures were set up in duplicate with CEE_{25} supplemented with formalinized *E. coli*,[4] and

[4] Growth from 18-hour cultures of *E. coli* was inoculated into nutrient broth in centrifuge bottles, each bottle containing 100 ml of culture fluid. These were incubated 24 hours at 37°, the cells harvested by centrifugation, and exposed to 7% formalin in 0.85% saline for a total time of approximately 2 hours. The killed cells were then washed in cold saline to remove the formaldehyde. Samples of the formalinized cells, after washing, were inoculated into various bacteriological media for sterility tests; all proved negative for the presence of viable bacteria. An amount of packed wet cells, equivalent to approximately 200 mg dry weight, was suspended in 20 ml CEE_{25}, and this mixture was used as the test medium.

with CEE_{25} alone. These were transferred 4 times at 48-hour intervals. On the tenth day, no difference could be detected between larval growth in the tubes with or without bacterial cells; most larvae were comparable to advanced lung stages in development at this time. These cultures were incubated with no further transfers being performed for a total cultivation period of 71 days. At this time, approximately 50% of the larvae were still alive in the tubes with *E. coli,* but showed no further advance in development from what had been encountered previously (Pl. I, Fig. 2). In the CEE_{25} alone, however, less than 1% of the larvae were alive. Formalinized *E. coli,* therefore, increased the survival rate, but did not stimulate growth beyond the third stage.

A considerable number of cultures were subsequently studied in which CEE was supplemented with various concentrations of agar, gelatin, rice powder, and whole rabbit blood singly and in various combinations. The increased viscosity of the medium due to the inclusion of agar or gelatin did not prove to be of any particular benefit. Growth in all these cultures could be correlated only with the presence of relatively high concentrations of CEE.

CEE_{50} was next supplemented with a casein hydrolysate and vitamin mixture at two different concentrations, and with the vitamin mixture alone. The following substances were added to CEE_{50}; the final concentration in the embryo extract is given in micrograms per ml.

Medium C. Acid-hydrolyzed casein,[5] 750; cystine, 1.5; tryptophan, 10; folic acid, riboflavin, 1.5 each; biotin, p-aminobenzoic acid, nicotinic acid, nicotinic acid amide, calcium pantothenate, pyridoxine hydrochloride, pyridoxal hydrochloride, thiamine hydrochloride, ascorbic acid, 3 each; B-12, 7.5; glutamine, 45; choline chloride, 6.

Medium D. All the supplements listed in medium C were added at twice the concentration to CEE_{50}, to prepare medium D.

Medium E. All supplements listed in medium C were added to CEE_{50} in the same concentration as given in C except for the omission of the casein hydrolysate, cystine, and tryptophan.

Cultures in each medium were set up in duplicate, and transfers were made at 24-hour intervals for a period of 10 days. The cultures then were maintained without transfer, but were examined periodically until day 31, when they were terminated.

In all media, larvae showed development comparable to at least mid- or late-lung stages. None progressed beyond these in either medium C or E. Approximately 65% of larvae from the former and 90% from the latter were living on day 31. Development in medium D, on the whole, was similar to the above, except that 5 larvae were comparable to early gut stages, reaching up to 1.6 mm in length (Pl. I, Fig. 4). It appeared then that the higher concentration of the amino acid-vitamin mixture had a slightly stimulatory effect on larval development.

Since it was obvious that growth factors qualitatively or quantitatively were still inadequate in the media tested, and that an amino acid-vitamin mixture enhanced development slightly, a further attempt was made to find natural products or their derivatives which might supply these and possibly other factors in adequate amounts. While these studies on the growth of the parasitic stages were being

[5] Bacto-casamino acids, Difco Co.

conducted, the nutritional requirements of the free-living stages of *N. muris* were concomitantly investigated. There was the possibility that information gained for one stage in development might provide a clue to the needs of the other developmental stages. It was found that the free-living stages could be grown in a mixture of sodium caseinate, yeast extract, and CEE. In this mixture, serum could be substituted for the CEE, and development of filariform larvae would still take place. With this information, these substances were then tested for their ability to support growth of the parasitic stages.

Cultures established in media F and G (Table I) were transferred once every 24 hours for a period of 7 days, and were finally terminated on days 10 and 11. At this time, approximately 95% of the larvae present in cultures F and G were alive and in excellent condition. Most larvae in F were comparable to early lung stages, while in G they had advanced to mid- to late lung stage in development. However, in each medium, a few worms were found undergoing the third molt; 4 in F and 6 in G. This was the first time in which molting had occurred in such low concentrations of embryo extract (10%, medium G), or in its absence (medium F).

TABLE. I.—*Components of media given in parts per 10 ml*

Medium	Caseinate,* 2%	Yeast extr.,** 2%	CEE_{50}	Horse serum
F	3.5	3.5	—	3.0
G	3.0	3.0	2.0	2.0
H	3.0	3.0	4.0	—
I	3.0	3.0	3.0	1.0

* *2% sodium caseinate plus cystine.* 4 grams of "Vitamin Free" Casein (Nutritional Biochemical Corp., Cleveland, Ohio) and 50 mg cystine were suspended in 150 ml distilled water. 1N NaOH was added until solution was complete (pH 11). The solution was then back-titrated to pH 7.1 using 1N HCl, and the volume adjusted to 200 ml with distilled water. It was sterilized by autoclaving.

** *2% yeast extract.* 2 grams of powder (Yeast Extract Powder, Nutritional Biochemical Corp.) were dissolved in double strength Tyrode Solution adjusted to pH 7.2. The solution was sterilized using a Selas 03 filter.

The high rate of survival and good condition of the worms suggested that continued transfer might eventually result in increased development for at least some of the organisms. The next cultures, which were prepared in duplicate, (media H and I, Table I) were transferred once every 48 hours. Cultures of medium H were terminated on day 34 and I on day 41.

In medium H at termination, on an average, 94% of the worms were alive. Most were comparable to mid- to late lung stages, but a small number of larvae had continued development. A few were in the third molt, 6 worms were variously sized fourth-stage males and females (Pl. IV, Fig. 11), and 2 male fifth-stage worms had appeared, one still being alive and in excellent condition. Although these 2 adult worms had fully formed bursae and normal spicules, the testis in each was still juvenile, no sperm being formed.

At termination of medium I cultures, 86% of the worms were alive, and a relatively larger number of advanced stages had developed. One hundred and seventy-six worms were recovered which were comparable to mid- to late lung stages; 10 were in the third molt; 38 were variously sized male (Pl. II, Fig. 5) and female fourth-stage worms; and 3 fifth-stage males and 1 female had appeared. The male adult worms had fully developed bursae and spicules, but the testes were immature. These worms measured approximately 2 mm in length. The adult female, 2.5 mm in length, contained eggs developing in the ovary, but none were in the uterus or

more terminal portions of the genital tract. All the fifth-stage worms appeared "stunted," although showing the normal morphological differentiation of young adults. The fourth- and fifth-stage worms were pink in color, which was presumably due to the presence of haemoglobin dissolved in the body fluid, comparable to the appearance of worms which develop in the rat.

These results indicated that serum had a definite enhancing effect on growth and development when incorporated into the caseinate, yeast extract, CEE mixture, and that continued transfer of such cultures over a prolonged period of time eventually gave rise to fifth-stage worms.

The following experiment was performed to repeat and extend the above findings. Table II presents the media investigated (J–Q), each of which was tested in duplicate tubes, transfers being performed once every 48 hours. Table III lists the number of worms in various stages found at the termination of the cultures.

TABLE II.—*Components of media given in parts per 10 ml*

Medium	CEE_{50}	Sodium caseinate* 2%	Trypticase** 2%	Trypticase† 5%	Yeast Extract* 2%	Liver Concentrate†† 2%	Horse serum	Rat serum	Rabbit serum
J	3	3	–	–	3	–	1	–	–
K	3	3	–	–	3	–	–	1	–
L	3	3	–	–	3	–	–	–	1
M	3	3	–	–	–	3	1	–	–
N	3	3	–	–	–	3	–	1	–
O	3	–	3	–	3	–	–	1	–
P	3	–	3	–	–	3	–	1	–
Q	3	–	–	3	3	–	–	1	–

* Prepared as given in footnote to Table I.
** Containing 1.6 mg percent cystine.
† Containing 4 mg percent cystine.
†† Preparation of 2% liver extract concentrate: Two grams of powder (Liver extract concentrate, 1:20; Nutritional Biochemical Corp., Cleveland, Ohio) were suspended in 100 ml double-strength Tyrode solution adjusted to pH 7.2 and extracted for approximately 1 hour at room temperature. It was initially clarified by centrifuging for 10 minutes at 1700 rpm. The supernatant was passed through filter paper with the aid of suction for further clarification, and the filtrate was sterilized using a Selas 03 filter.

The results are still too preliminary in nature to allow for definite conclusions concerning differences between yeast and liver extracts, the relative merits of rat, rabbit, or horse serums, etc. All these will be investigated in greater detail under more precise test conditions, since the cultures in this series were not all terminated at the same time. Of importance, however, is confirmation of the previous experiment that adult worms would develop in non-cellular, non-living media.

Description of stages in culture

From an examination of Table III, it can be seen that fifth-stage worms appeared in all media except J, M, and Q. In J and M, the lack of adults and the relatively small number of fourth-stage worms can probably be related to the batch of horse serum used for this particular experiment. Although adult worms did not develop in Q, this medium did contain fairly large numbers of fourth-stage organisms. In all other media where fourth- and fifth-stage worms did develop, little difference could be noted in comparing the morphological appearance of nematodes in the same stage of development, but derived from different cultures. However, in medium N, the worms tended to appear more robust, and these were the only cultures in which sexually mature males and females were found. Accordingly, a

general description of development will be presented for this series of cultures (J–Q), pointing out differences only where important ones occurred.

The almost incessant activity which was manifested in all these media by worms in various stages, was extremely striking. The third-stage larvae were mostly "serpentine" and progressive in their movements, but fourth- and fifth-stage worms would knot, coil and uncoil, and interpose rapid progressive motion in which the nematodes would propel themselves off the floor of the tube and swim forward.

Growth during the first week was gradual, but by the seventh day, a few worms in almost all cultures had begun the third molt, the loosened cuticles being plainly visible (Pl. I, Fig. 3). The very young fourth-stage worm is most easily distinguished from a fully mature third stage by the appearance of the cephalic cuticular expansion. Under the low power used for the examination of larvae in roller tubes, this delicate structure was not easily seen. It was thus difficult, once molting had

TABLE III.—*The numbers of worms in various stages of development found at termination* of cultures in the media J to Q, listed in Table II*

Medium	Stages of worms**							Total Nos. of worms
	a	b	c	d	e	f	g	
J	5	119	13	3	0	0	0	140
K	1	153	19	49	2	8	0	232
L	3	170	9	31	1	6	0	220
M	1	163	6	16	1	0	0	187
N	0	147	12	27	6	16	8	216
O	3	134	13	44	4	3	0	201
P	2	80	20	63	3	7	0	175
Q	0	79	21	66	0	0	0	166

* A few adult worms were removed from some of these cultures for examination before the cultures were terminated; these are included in the table.

** a—Slight growth beyond filariform
b—Middle to late lung stages
c—In third molt
d—Fourth stage
e—In fourth molt
f—Fifth stage, sexually immature
g—Fifth stage, sexually mature

occurred, to identify with certainty the newly emerged fourth-stage worm. However, by the tenth day, sufficient growth of some of the worms had taken place, with enough differentiation of internal structure, so that the presence of fourth-stage worms could be definitely established.

The development of the bursa of the male could be followed with a fair degree of clarity, from the early swelling of the posterior, to the formation of the bursal lobes and rays (Pl. II, Figs. 6, 7, 8). The differentiation of the vagina, ovijector, uterus, and ovary could similarly be seen in the female. The first appearance of a fifth-stage male worm with a completely developed bursa which was observed freely expanding and contracting, was on day 17 in medium N, and on day 19 a fully mature male had appeared with sperm filling the seminal vesicle. Adult males began to be found in other media at about this time, also. The adult male worms ranged from 1.8 to 2.4 mm in length (Pl. III, Fig. 9).

Although many fifth-stage male worms developed in the various cultures, most did not become sexually mature, as judged by the fact that the testis did not produce sperm. The external sex organs, however, were morphologically normal. The bursae of many worms were carefully checked and all showed a configuration of bursal lobes and rays which was similar in all details to that described and figured by Yokogawa (1920), and also agreed satisfactorily with specimens we recovered

from laboratory-infected rats. The spicules and gubernacula were also normal in appearance. These external sex organs, however, were small but seemed proportionately correct, inasmuch as the worms from culture were undersized when compared to those recovered from the rat intestine.

The male worms which contained sperm filling the seminal vesicle were crushed in saline, and the released sperm were carefully examined and measured. The sperm cell of *N. muris* consists of a headpiece and tail, and is apparently pseudoflagellate, since no motion of the sperm was ever detected. The sperm from cultured worms were exactly comparable in all morphological details and size to those recovered from male worms which had developed in the intestine of the rat; they were approximately 13 to 17 microns in total length (Pl. III, Fig. 10).

The first appearance of fifth-stage female worms was more difficult to establish with certainty than was that of the male, since the differentiation between the fourth- and fifth-stage female is not as easily recognizable under low power. However, unmistakable fifth-stage females were found beginning with day 20 (Pl. IV, Fig. 12), and these were verified by isolating them from the culture tube so that they could be examined more critically on a slide. The fifth-stage female worms which developed in the cultures varied from approximately 2.1 to 2.5 mm in length.

Most such female worms which developed were not considered sexually mature. Many showed an apparently well developed and completed genital system, but eggs had not yet appeared in the uterus (Pl. IV, Fig. 13). In others, egg formation was either abnormal, or some part of the internal sex organs was incomplete or malformed. For instance, eggs were produced which were misshapen jelly-like masses extruded into the vagina, still attached one to another. In a few worms, the posterior portion of the genital tract appeared normal in development, but the ovary had not fully matured. Three worms, however, were found comparable to sexually mature adult females from rats; their genital tracts were normal and eggs were present in the uterus (Pl. IV, Fig. 14). The eggs, however, appeared infertile and no sperm were seen in the seminal receptacles or elsewhere in the genital tract. All these female worms were considered undersized when compared to normal adult worms recovered from the rat intestine.

As already indicated, the developmental sequence of fourth- and fifth-stage worms was not uniform, even in the best cultures obtained. For example, fifth-stage male worms began to appear in medium N on day 17. Yet, other male worms in this medium did not transform to the fifth stage until approximately day 35.

DISCUSSION

The results of the present study demonstrate that it is possible to cultivate a parasitic nematode of a vertebrate from egg to adult under axenic conditions *in vitro*. The successful use of antibiotics to render nematode cultures free from bacteria has been reported by several investigators (Dougherty and Calhoun, 1948a; Weinstein, 1949; Epps, Weiner and Bueding, 1950). The inclusion of penicillin and streptomycin in the media greatly facilitated the preparation of axenic cultures from filariform larvae. As a result, it was no longer found necessary to carry out the complicated settlings and many washes described by Glaser and Stoll (1940) for ridding *H. contortus* larvae of bacteria in their gut, which was an absolutely necessary procedure prior to the discovery of antibiotics.

The preliminary treatment of the *N. muris* larval suspension with hypochlorite solution, followed by several washings in sterile saline, drastically reduced the number of bacteria associated with the nematodes. If we can judge from our experience with the sterilization of eggs of *N. muris* recovered by salt flotation from rat feces, the larval surfaces were probably rendered sterile, since we consistently obtained sterile egg suspensions by treating them with the same concentration of hypochlorite as was used for the larvae. At least some of the larvae contained small numbers of bacteria in the gut lumen, which did not come in contact with the hypochlorite, and thus escaped destruction. With the ingestion of the experimental medium, these bacteria were exposed to sufficiently high concentrations of penicillin and streptomycin to result in their death.

If the larvae were exposed for much more than 20 minutes to the hypochlorite solution, they began to show vacuolization, particularly around the esophagus. However, there was no indication of any damage resulting from the standard exposure, nor was there any reason to suspect that the antibiotics used were at all inhibitory.

The complexity of the media necessary to initiate and maintain development does not permit an analysis of specific growth factors at this time. It is apparent, however, that embryo extract alone in high concentration will initiate parasitic development and support the growth of the larvae to an advanced lung stage. Very little growth occurs beyond this point in this medium, and development of the larvae then remains suspended at this stage, even though transfers are continued. Fourth- and fifth-stage worms appeared to a greater or lesser degree, only when embryo extract was supplemented with various substances such as mixtures of amino acids and vitamins, serum, or extracts of complex biological material like yeast or liver, known to contain high concentrations of vitamins and possibly cofactors. At present, a combination of 4 groups of substances will support the development of adult worms: (1) caseinate or a casein digest, (2) filtrate of an aqueous extract of yeast or liver powder, (3) mammalian serum, and (4) chick embryo extract. Of particular interest, however, is the fact that sexually mature adult worms developed only in the medium composed of caseinate, liver extract, rat serum, and embryo extract.

Although it is possible that serum derived from the natural host (in this case, rat) may prove to have a more salutary effect than that derived from some other source, it may prove that the method of processing and conditions of storage can also be of importance. For instance, in the first experiment using horse serum (medium I, Table I) a considerably greater number of fourth-stage, as well as a few fifth-stage, worms developed as compared to the last experiment (media J and M, Table III). In the former, the horse serum had been processed cold and stored at $-20°$, while in the latter experiment it had been obtained from a commercial source and was not stored frozen.

The size of the adult worms which developed in the cultures was definitely smaller than that of worms recovered from the normal host, the wild rat, *Epimys rattus norvegicus*. Yokogawa (1920) determined the length of fixed specimens, which were slightly shrunken, and found the males to range from 3 to 4 mm and the females from 4 to 6 mm. Lindquist (1950b) measured fixed specimens from the hooded laboratory rat and found the males to range from 2.9 to 4.0 mm (mean,

3.4 mm), while the females ranged from 3.1 to 5.0 mm (mean, 4.2 mm). Weinstein (1955) measured living specimens from the Sprague-Dawley laboratory rat and reported the males to range from 2.3 to 4.4 mm (mean, 3.5 mm) and the females from 3.4 to 6.0 mm (mean, 4.6 mm). The male adult worms recovered from cultures ranged from 1.8 to 2.4 mm, while the females ranged from 2.1 to 2.5 mm.

This apparent "stunting" of worms in culture is of considerable interest, when compared to the reports of stunting of *N. muris* adults in both abnormal hosts and in immunized normal hosts. The host range of *N. muris* is very limited. In hosts other than *E. norvegicus* and the laboratory rat, development of larvae to the adult stage occurs only in low percentage, and the prepatent period is usually extended. Porter (1935b) reported infection of the laboratory mouse, Lindquist (1950a, b) the cotton rat and hamster, and Thorson (1953a) the rabbit. Although larvae migrated to the lungs of the deer mouse and the guinea pig, and showed evidence of growth, adult worms did not develop in these hosts (Porter, 1935c; Lindquist, 1950b). Length measurements of adult worms in abnormal hosts are available only for the cotton rat. Lindquist (1950b) found the males to range from 1.7 to 3.0 mm (mean, 2.1 mm), and the females from 1.7 to 3.4 mm (mean, 2.4 mm). Similar stunting of adult worms occurs in immunized laboratory rats (Chandler, 1935; Taliaferro and Sarles, 1939). Weinstein (1955) reported that in such immunized animals, the males averaged 2.6 mm, and the females 2.8 mm in length.

This spectrum of the development of *N. muris* in nature, ranging from only lung stages in the deer mouse through stunted though sexually mature adults in the cotton rat and immunized laboratory rat, to fully developed worms in *Epimys* and the normal laboratory rat, has its counterpart in the development encountered *in vitro*. In CEE_{50} alone, the larvae reached the late lung stage and remained in this condition, which appeared comparable to the deer mouse end of the spectrum. When CEE was supplemented with various other nutrients, sexually mature though stunted worms developed, which seemed to correspond fairly reasonably to growth in the cotton rat. The level of development encountered in the normal host is yet to be attained in culture. These data suggest that one of the factors involved in the phenomenon called "host specificity" may be the nutrients available to the nematode at a particular time in its cycle. Development to the advanced lung stage appears to be fairly non-specific, induced by whole blood. But beyond this point, particular nutrients probably have to be available qualitatively and quantitatively to continue growth through the fourth and fifth stages. Animals which supply these may have the potential to act as host for the parasite. Other factors which are involved in resistance, such as cellular response, may complicate this picture considerably.

Stunting of worms in the immunized animal is possibly also related to a nutritional problem, although this is still not completely resolved. Chandler (1937) has suggested that in such animals, anti-enzymes develop which inhibit the nutrition of the worm. Sarles (1938) has demonstrated precipitate formation in immune serum which plugs the oral aperture of the worms and appears to block feeding. Thorson (1953b) collected secretions and excretions of *N. muris* larvae which possessed lipolytic activity. Upon incubating these preparations with immune serum, the lipolytic activity was inhibited. He (1956) subsequently demonstrated inhibition by immune serum of proteolytic activity of esophageal extracts of adult hook-

worms. The use of *in vitro* cultures may provide a tool with which the effects of these phenomena on actual growth and differentiation may be studied.

It is also possible that naturally occurring inhibitory substances are present in certain tissues which may adversely influence worm development, when these are prepared as homogenates. Weinstein (1949) and Dougherty (1951) have presented some evidence for this.

The above discussion concerning nutritional factors in the spectrum of host specificity is, of course, highly speculative in nature. It points, however, to some of the fundamental problems of nematode development which may be investigated by *in vitro* culture methods, free from the complicating association of the host, and which may ultimately shed considerable light on the factors influencing growth and differentiation *in vivo*.

The nutritional composition of a culture, although undoubtedly of major importance, is not the only factor involved in differentiation of the parasitic stages. Physical factors such as temperature play an important role, and because of their obviousness are sometimes overlooked. As an example, axenic development of the free-living stages of *N. muris* will occur in CEE_{50} at approximately 27°. The filariform larvae which develop in such cultures show no further morphological changes when maintained in the extract at this temperature over a period of many weeks. However, development of the larvae comparable to advanced lung stages in the rat will occur rapidly in CEE_{50} alone, merely by raising the temperature to 37.5° C. Similarly, changes in the gas phase, physical consistency of the medium, or just the *timing* at which particular nutrients are supplied to a worm may be factors influencing its development. A nematode which normally resides and feeds in many different organs of the host during its course of development, ranging from the lungs to the lumen of the small intestine, undoubtedly encounters many changing nutritional and physical conditions which may affect its growth. Duplicating these factors in their correct sequence and in time may perhaps be necessary for the successful cultivation of many parasitic helminths.

Although a few sexually mature male and female worms were present in the same culture simultaneously, fertilization apparently did not occur. With such small numbers distributed in a relatively large volume, however, the chance for one worm to encounter another was slim. It is felt that with continued improvement of the media and of methods of handling the cultures, higher yields of sexually mature adult worms will be obtained. This will greatly increase the probability that mating and fertilization will occur, with the resultant production of viable eggs. Once this is achieved, it is hoped that *N. muris* may be maintained indefinitely *in vitro*, if desired.

SUMMARY

1. *Nippostrongylus muris* has been successfully cultured in cell-free media under axenic conditions *in vitro* through the free-living and the parasitic cycles.

2. Development comparable to advanced lung stages in the rat has been obtained in 50% chick embryo extract alone. Further growth and differentiation comparable to fourth- and fifth-stage worms in the gut occurred only when embryo extract was supplemented in a variety of ways.

3. Fifth-stage worms were obtained in media combining the following compo-

nents: (a) caseinate or casein hydrolysate, (b) filtrate of powdered yeast or liver extract, (c) chick embryo extract, and (d) mammalian serum.

4. Sexually mature males containing sperm and females containing eggs have so far appeared only in a medium containing caseinate, liver filtrate, chick embryo extract, and rat serum. The adult worms were morphologically normal, although stunted in growth; the males ranged from 1.8 to 2.4 mm, and the females from 2.1 to 2.5 mm in length.

5. It has taken at least 3 to 4 times longer for fifth-stage worms to develop in culture, than in the rat.

6. Mating has apparently not occurred, due probably to the small numbers of adult worms which have appeared at any one time.

7. Roller tube cultures have proved to be considerably more advantageous than flask cultures. They are easier to handle, and allow continual observation of the organisms in the media.

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Explanation of Plates

The following photographs, other than Figure 1, represent organisms in various stages of development, corresponding to the parasitic cycle of *N. muris*. Although taken from a variety of media, they illustrate fairly typically the progression in stages of development as seen in individual cultures which supported growth of worms to the adult stage, e.g., in medium N, Table II.

Fig. 1. Ensheathed filariform larva cultured axenically from the egg stage in 44 percent chick embryo extract. ×158.

Fig. 2. Larva corresponding to advanced lung stage. Note typical development of deep brown pigmentation of gut granules; CEE_{25} plus formalinized *E. coli*, day 71. ×158.

Fig. 3. Larva going through third (first parasitic) molt; medium P, Table II, day 27. ×158.

Fig. 4. Young fourth stage male worm; CEE_{50} supplemented with casein hydrolysate and vitamin mixture (medium D) day 12. ×158.

Fig. 5. Advanced fourth-stage male worm corresponding to those seen at termination of the culture; developing bursal rays and spicules are clearly visible, body fluid is pink; medium I, Table I, day 26. ×158.

Fig. 6. Male worm molting to fifth stage (fourth molt); medium K, Table II, day 27. ×158.

Fig. 7. Posterior of sexually mature fifth-stage male worm; medium N, Table II, day 38. ×158.

Fig. 8. Posterior of fifth-stage male worm showing details of spicules, gubernaculum, and the typical asymmetry of the bursal lobes and rays. Vacuolization was the result of coverslip pressure; medium L, Table II, day 27. ×333.

Fig. 9. Adult male worm, sexually mature. Note coiled testis, sperm filling seminal vesicle, characteristically "vacuolated" appearance of glandular tissue surrounding the ejaculatory duct, and the protruded spicules; medium N, Table II, day 38. This is a composite picture made by photographing the anterior and posterior of the worm separately, and then combining the two photographs. ×158.

Fig. 10. Sperm cells liberated from a crushed adult male worm; medium N, Table II, day 19. ×455.

Fig. 11. Young fourth-stage female worm. Note early differentiation of vaginal area; medium H, Table I, day 33. ×158.

Fig. 12. Female worm undergoing fourth molt to the fifth stage; medium L, Table II, day 27. ×333.

Fig. 13. Anterior of fifth-stage female worm not yet completely sexually mature, but note well developed normal ovary producing a large number of eggs; medium K, Table II, day 27. ×158.

Fig. 14. Posterior of sexually mature adult female. Note eggs in uterus, the characteristically thickened posterior wall of the ovijector, and the folded and twisted inner surface of the vagina; medium N, Table II, day 28. ×158.

PLATE I

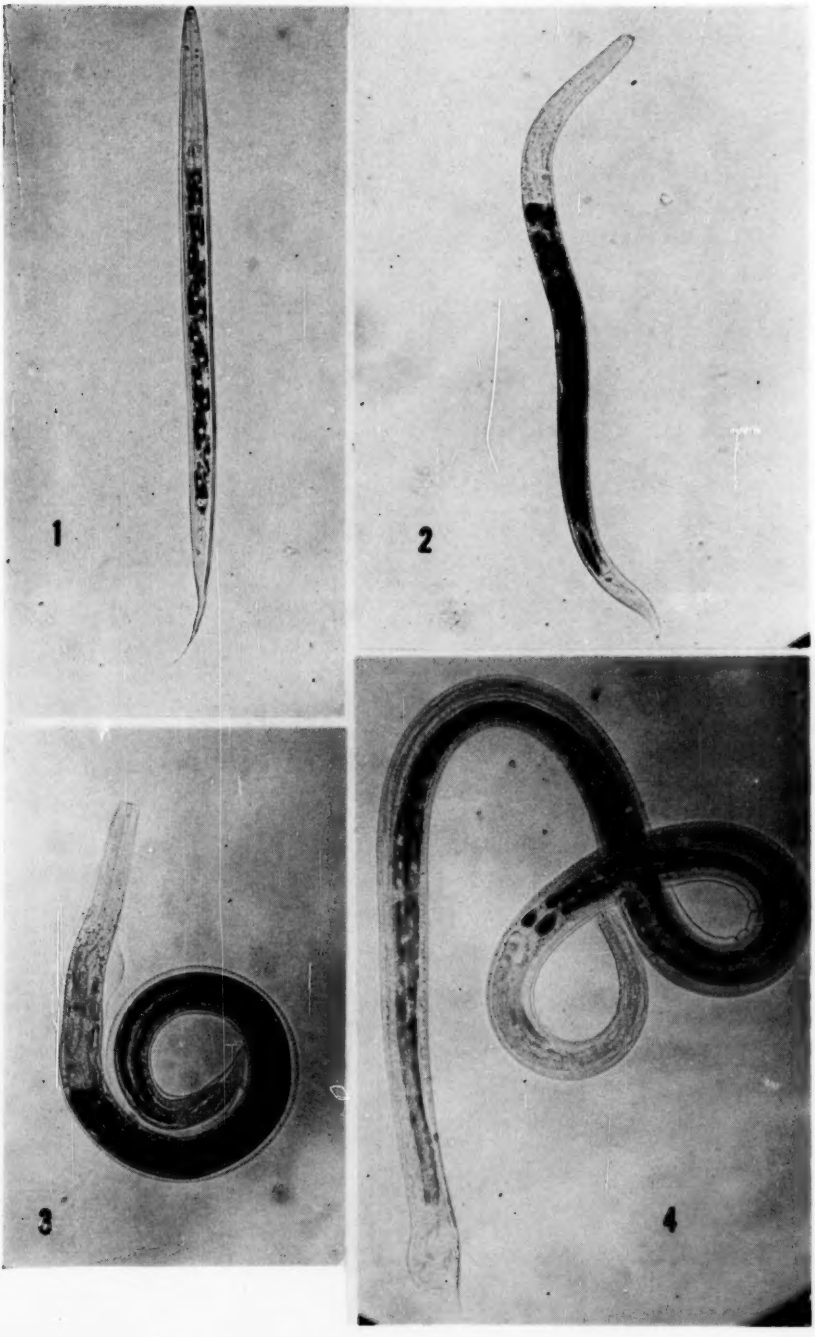
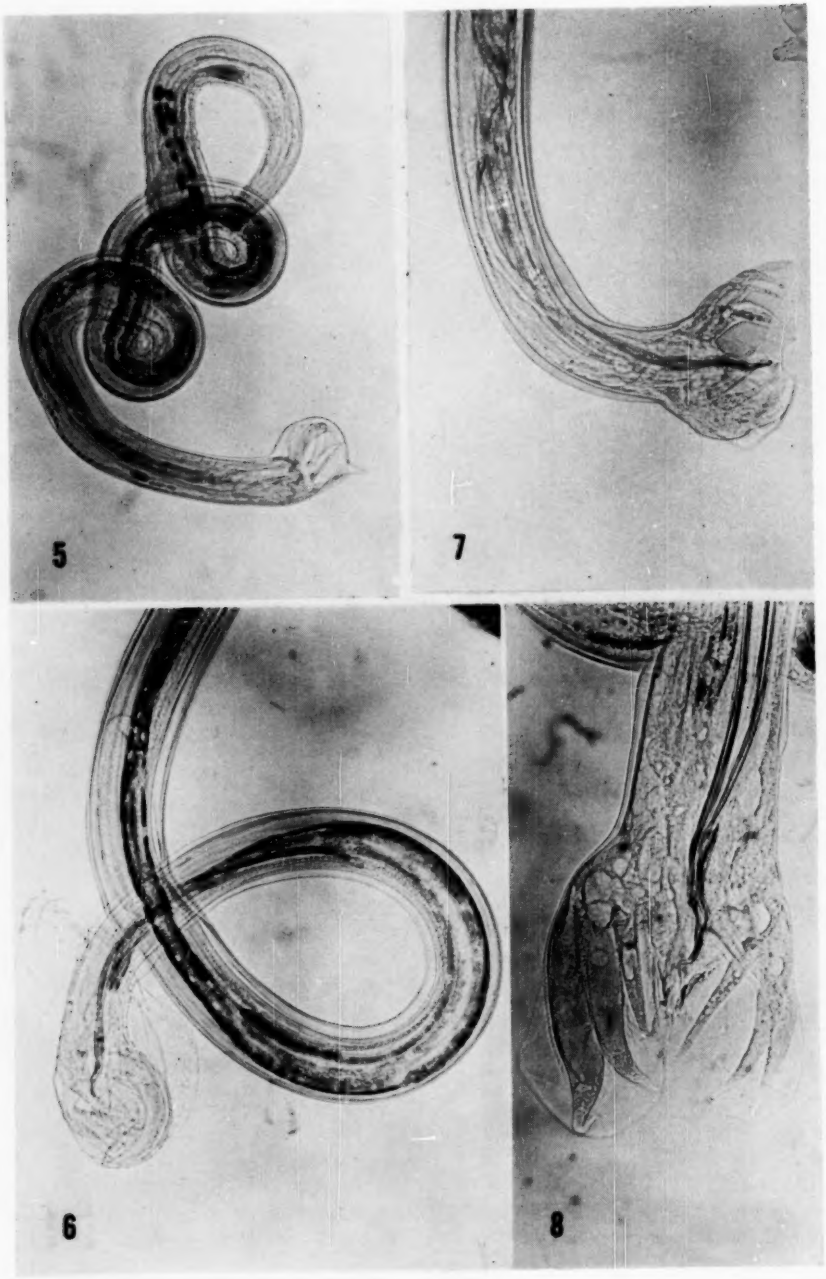


PLATE II



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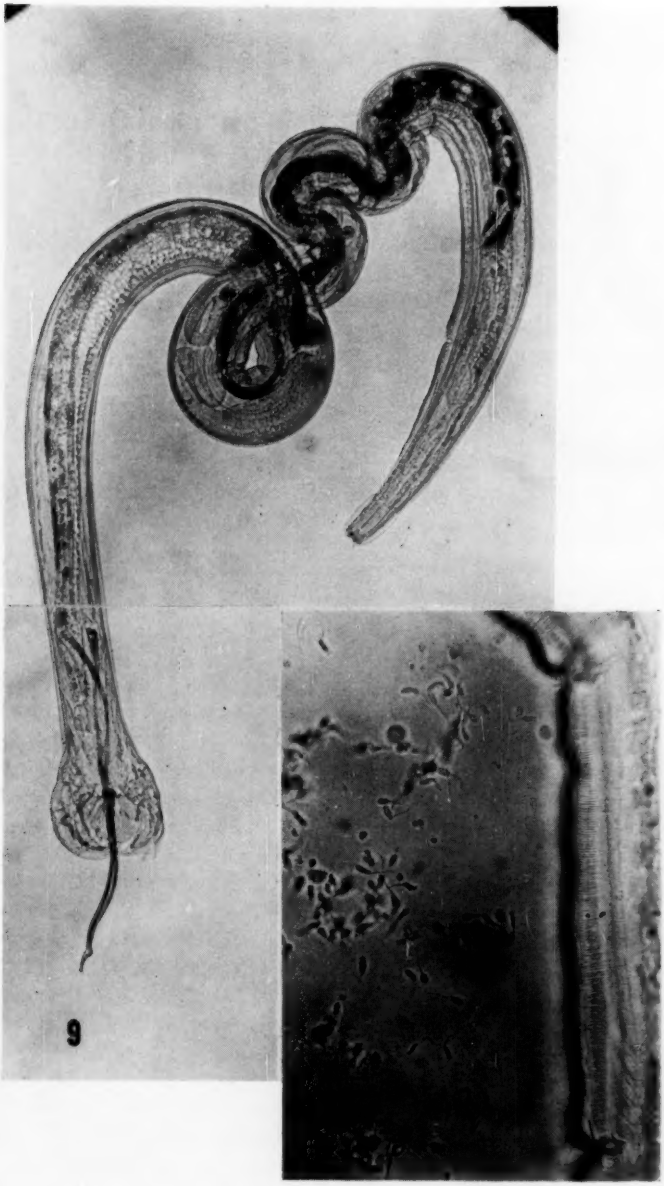

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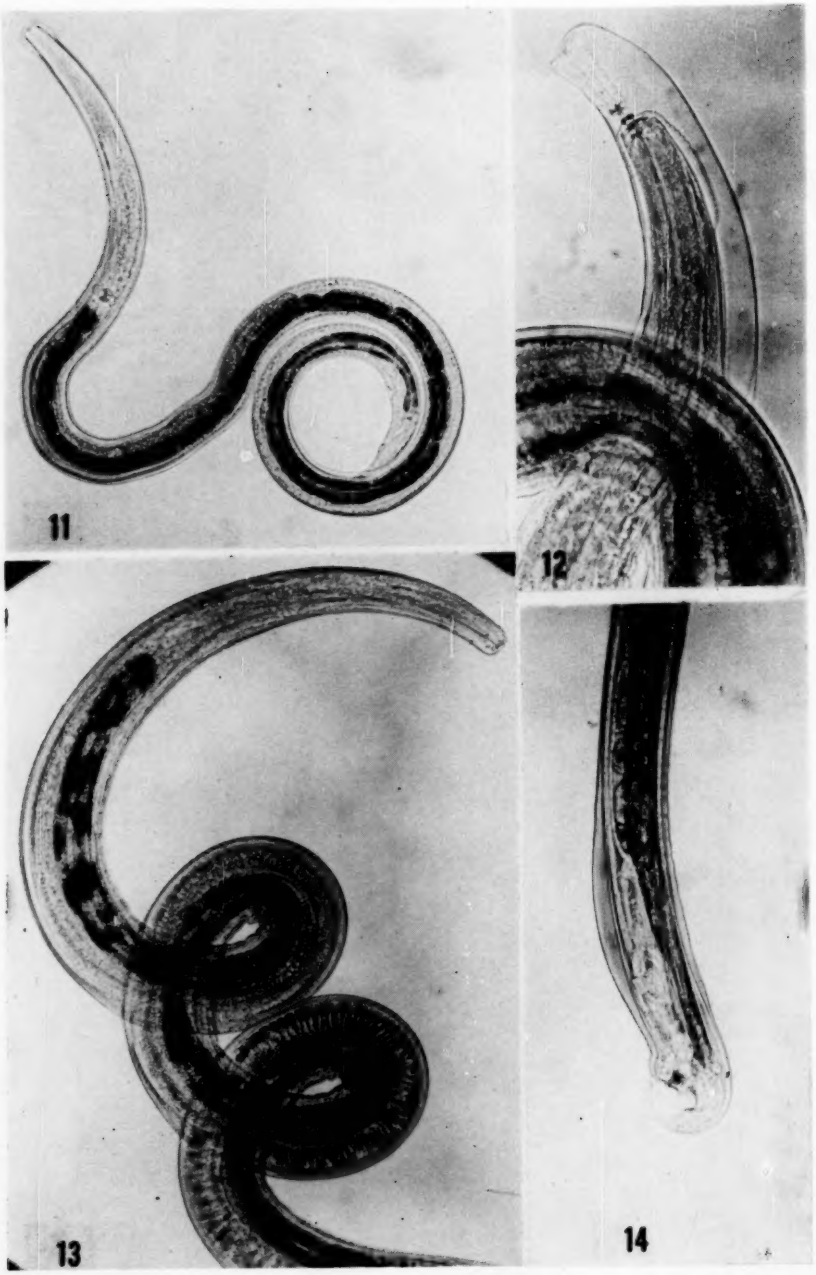

STUDIES ON THE SEROLOGY OF TRICHINOSIS WITH HEMAGGLUTINATION, AGAR DIFFUSION TESTS AND PRECIPITIN RING TESTS *

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Serological tests are valuable aids in the diagnosis of trichinosis. The need exists however for a serological technique that will detect natural infections in humans and in swine after exposure. In addition to the precipitin, complement-fixation and skin tests (Bozicevich, 1952), a flocculation test (Bozicevich, *et al.*, 1951, Thomas, *et al.*, 1953, Norman, *et al.*, 1955 and Sadun and Norman, 1955), and an agglutination test (Thompkins, *et al.*, 1952 and Campbell, 1953) have been reported. We have used a hemagglutination test for the detection of antibody in the sera of rabbits and rats experimentally infected with *Trichinella spiralis*. The sensitivity of the hemagglutination test was compared to the precipitin ring test in their respective abilities to detect antibody early in infection. An acid-soluble larval protein antigen (Melcher, 1943) was used and the number of its antigenic components was determined by use of an agar double diffusion technique. The results of these tests are reported in this paper.

MATERIAL AND METHODS

Larvae of *T. spiralis* were isolated from infected rat muscle by digestion in a 1% pepsin and 0.7% HCl mixture for one hour at 37° C. Washed larvae were then fed by stomach tube to several experimental hosts. Three rabbits in group A (R29, R30, R31) received 12,000, 18,000, and 24,000 larvae, respectively. Two rabbits in group B (R1 and R2) received 13,000 larvae each. Twenty-two rats were infected as follows: 10 animals received 1000 larvae, 3 animals 1200 larvae, 3 animals 2500 larvae, 4 animals 5000 larvae and 2 animals 6000 larvae. Prior to infection all animals were bled for control serum. Following exposure, rabbits in group A were bled twice weekly from an ear vein for 61 days (47 days for group B), and rats once a week intracardially for 14–43 days. Blood obtained from individual groups of rats was pooled. The collected sample was kept at room temperature for one hour, the clot separated from the sides of the glass tube with a wood applicator stick and stored at 4° C. The following morning after centrifugation, the serum was heated at 56° C for 30 minutes and without further treatment placed in the deep freeze at –20° C. The sera of rabbits R1 and R2 were obtained from an experiment by the senior author and Dr. C. M. Coker. We wish to thank Dr. Coker for collecting the sera and for his permission to use them in this study.

Fifty human sera were obtained from the serological laboratory of a local hospital. Two sera from patients with clinical trichinosis were obtained from Dr. W. G. Sawitz and 19 sera from patients with schistosomiasis from Colonel E. M. Chaffee.

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The antigen used in the hemagglutination test was Melcher's acid-soluble larval protein fraction (Melcher, 1943). 270 mg dried larvae were extracted in a Soxhlet apparatus with petroleum ether for one week at 52° C. After drying the extracted larvae in a vacuum desiccator, the material was mixed and stored overnight at 4° C with 20 cc of a borate buffer (pH 8.2). Following centrifugation after overnight contact with the borate buffer the sediment was discarded and the pH of the supernatant adjusted to 4.2 with .2 N HCl. The acid insoluble precipitate which comes out of solution at the low pH was separated by centrifugation and discarded. The clear supernatant was used as antigen and estimated to be approximately a 1:100 dilution. Unless otherwise stated, Melcher's antigen used in all tests is this 1:100 dilution.

The hemagglutination test as outlined by Boyden (1951) was followed with minor modifications. Sheep red cells [1] in Alsever's solution were washed three times in a phosphate saline buffer (pH 7.2) and adjusted to a 2.5% suspension with the same buffered saline. An equal volume of 1:20,000 tannic acid in buffered saline was added and the mixture incubated at 37° C for 10 minutes. After washing the cells free of excess tannic acid with buffered saline, for each volume of cells five volumes of a 1:1600 solution of Melcher's antigen was added and the cells incubated at room temperature (22°–26° C) for 15 minutes. The diluent for the antigen mixtures was buffered saline. Two ml of normal rabbit serum, 1:250 in buffered saline, was added after 15 minutes and the mixture centrifuged at 1000 rpm for 3 minutes. The packed cells were then resuspended with 1:250 normal rabbit serum into a 2% suspension and used as antigen. Two-fold serial dilutions of test sera were made in 1:100 normal rabbit serum starting at 1:10 in tubes 10×70 mm. One drop of antigen-treated cells was added to 0.5 cc diluted serum with an Ives dropping pipette.

The pattern of the red cells on the bottom of the tube was read after 2 hours and on the following morning after remaining at room temperature or at 4° C. A serum dilution was recorded as showing 4+ agglutination when the cells were evenly distributed on the bottom of the tube. For each serum at least three tests were made. Rabbit sera in group A were tested with the same batch of cells on 4/14/55, 4/27/55 and 5/4/55. The titers obtained in these three tests are listed in Table I and the results of test 4/27/55 (Table I) for rabbit and 5/7/55 for rat sera were selected arbitrarily and plotted in Figures 1 & 2. Each test was controlled with a reagent control [normal rabbit serum (1:100) plus tannic acid treated and antigen coated cells], tannic acid control (tannic acid treated cells tested with one normal and one immune serum). Before each large series of tests one normal and one infected serum was tested with normal sheep cells and sheep cells treated with antigen.

Ring tests in 3 mm tubes (O.D.) with .02 ml of serum overlaid with .02 ml antigen were read after 5 and 60 minutes. Titrations were made by diluting the antigen with saline and testing against full strength serum. The antigens used were Melcher's antigen and a saline extract of trichina larvae prepared by homogenizing 0.5 cc packed living larvae with 5 cc saline. Sera from cases of schistosomiasis were also tested with saline antigens (1:100) prepared from lyophilized cercariae and adult worms of *Schistosoma mansoni.*

[1] Sheep cells were obtained from Cappel Laboratories, West Chester, Pa.

Double diffusion tests for the antigenic analysis of Melcher's antigen were made in 3-mm tubes (O.D.) after the technique of Oakley and Fullthorpe, (1953), modified by Preer (unpublished). Tubes were prepared by coating 16-cm lengths of 3-mm tubing with 0.1% agar and then cutting pieces 4 cm in length and sealing one end with a flame. With a blunted 2-inch 25-gauge hypodermic needle attached to a 0.25-ml syringe, 0.01 ml antiserum was dispensed into a tube followed by 0.01 ml of liquid 0.6% washed agar. After allowing the agar to cool and solidify, 0.01 ml Melcher's antigen was added. Agar was prepared with M/15 phosphate buffer, (pH 7.0) with 0.01% merthiolate added. Titrations of antiserum were made by diluting the antiserum with normal serum and the antigen with saline. Tests were usually read after 24 hours and for 3–6 consecutive days. Drawings of the bands which appeared in the agar were made daily by holding a tube against a millimeter rule and measuring the distance of the band from the antigen or serum meniscus.

EXPERIMENTAL RESULTS

The sera of all experimentally infected rabbits were shown to have antibody by hemagglutination. This antibody was detected 6 days after infection in two rabbits and by 15 days in the remaining 3 animals. For approximately 30–40 days following infection the antibody titer increased in infected animals (Fig. 1 and Table I).

For hemagglutination tests made on 4/27/55 (Table I and Fig. 1) rabbit R31 infected with 24,000 larvae showed a rapid increase in titer from 1:40 to 1:2560 between 10 and 21 days (end of experiment) after infection. Rabbit R30 infected

TABLE I—*Hemagglutination titers obtained in three comparative determinations with sera of animals infected with* Trichinella spiralis

Day After Infection	RABBIT SERA								
	Rabbit R29 Hemagglutination test			Rabbit R30 Hemagglutination test			Rabbit R31 Hemagglutination test		
	4/14/55	4/27/55	5/4/55	4/14/55	4/27/55	5/4/55	4/14/55	4/27/55	5/4/55
0	0	0	0	0	0	0	0	0	0
6	320	160	40	0	0	0	0	0	0
10	640	640	160	80	20	160	0	40	40
15	320	640	80	80	20	20	0	1280	0
17	320	320	80	80	160	160	320	1280	80
21	640	320	160	80	160	20	320	2560	320
23	1280	160	80	1280	160	1280			
28		640	640	2560	160	320			
31		5120	640	2560	1280	640			
35		10240	10240	1280	1280	160			
38		5120	2560	1280	640	1280			
42		2560	2560	1280	640	5120			
57		640	1280		2560	1280			
63		1280	1280			1280			

	RAT SERA								
	5000 larvae[1] Hemagglutination test			2500 larvae[1] Hemagglutination test			1200 larvae[1] Hemagglutination test		
	4/16/55	5/7/55	5/11/55	4/16/55	5/7/55	5/11/55	4/16/55	5/7/55	5/11/55
0	0	0	0	0	0	0	0	0	0
6 (5)[2]	0	0	0	0	0	0	1280	0	0
15 (14)	1280	0	0	80	80	0	1280	160	0
23 (20)	1280	160	320	640	640	80	5120	160	160
25 (22)	1280	1280	640	640	320	40	320	320	80
31 (30)	5120	2560	1280	640	320	160	1280	640	80
39 (37)	5120	1280					1280	80	80
46	5120	5120		320	320	160			

[1] Infective dose for group of rats.

[2] Figures in parentheses indicate the day of bleeding for rats infected with 1200 larvae.

with 18,000 larvae manifested an increase in titer from 1 : 20 to 1 : 1280 between 20 and 30 days after infection. R29 infected with 12,000 larvae showed an initial rise in titer to 1 : 640 by day 10 which declined to 1 : 160 on day 23 and rose rapidly from 1 : 160 to 1 : 10240 between 23 and 35 days after infection (Fig. 1). Both rabbits R1 and R2 each receiving 13,000 larvae, showed a steady rise in titer from 20–40 days after infection (Fig. 1). The antibody titer after reaching its maximum at approximately 35 days after infection declined slightly. The hemagglutination titers of individual sera declined over a period of three weeks (Table I). In general, variation between replicate runs differed as little as 2 or 3 dilution tubes. The general nature of the antibody curve for individual rabbits was not altered by indi-

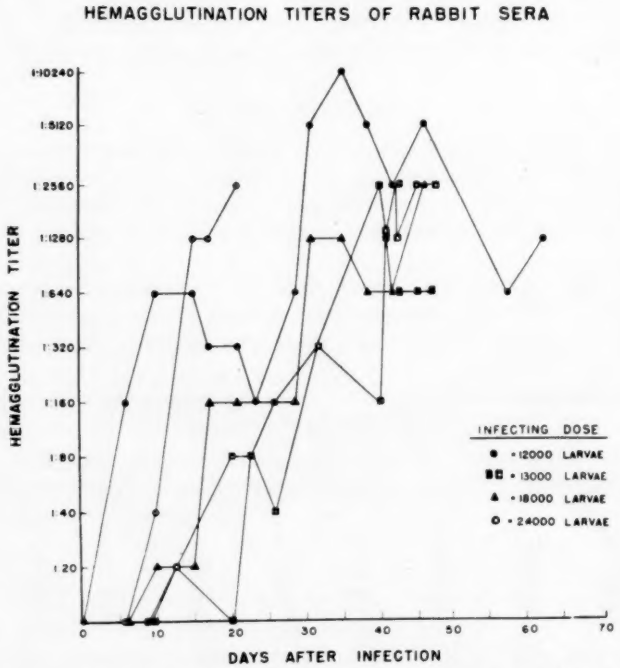

FIG. 1. Hemagglutination titers of five rabbit sera after experimental infection with *Trichinella spiralis.*

vidual variations between tests. Titration of two rabbit sera 4 months after infection showed a titer of 1 : 1280 indicating that the hemagglutination titer may be maintained at a fairly constant level for several months.

Titration curves with pooled rat sera are shown in Figure 2. Differences in antibody titers were noted with sera of rats receiving large, medium, or low infecting doses. Sera from animals infected with 5000 larvae showed the highest titers; those which received 1200 larvae, the lowest, while rates infected with 2500 larvae occupied an intermediate position (Fig. 2 and Table I). Hemagglutination was first detected 15 days after infection and appeared first in the sera of animals receiving the fewer inoculating doses (Table I test 5/7/55). Ten rats infected with

1000 larvae and bled for 14 days and two rats infected with 6000 larvae and bled for 11 days were negative for agglutinins.

Two human trichinosis sera were positive by hemagglutination with titers 1 : 160 and 1 : 1280 for one serum and 1 : 40 and 1 : 320 for the second. Thirty-one normal human sera were tested. One serum (tested twice) was positive with a titer of 1 : 20 and 1 : 40. Two sera of schistosomiasis patients that showed questionable positive ring tests with Melcher's antigen gave titers of 1 : 40 and 1 : 80 by hemagglutination.

Ring tests were made with the sera of rabbits in group A. Rabbit R29 which showed a positive hemagglutination titer in 6 days became positive with the pre-

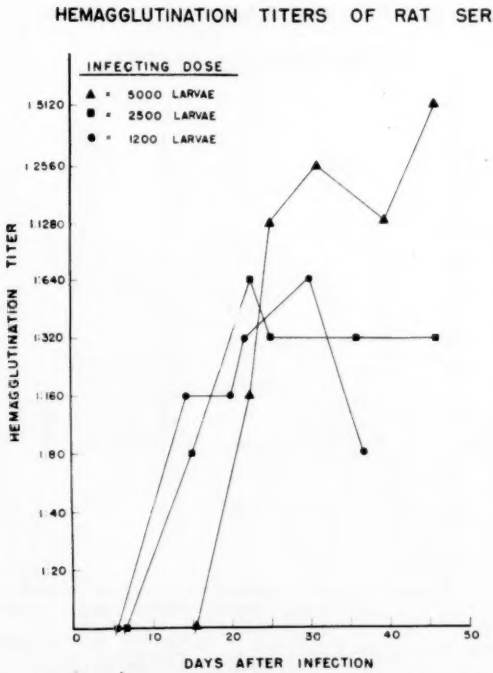

FIG. 2. Hemagglutination titers of pooled rat sera after experimental infection with *Trichinella spiralis*.

cipitin ring test with Melcher's antigen only 21 days after infection. With a saline extract of larvae as antigen the ring test became positive 42 days after infection. R30 showed a positive ring test on day 30 after infection with Melcher's antigen and was negative with the saline antigen. R31 which was killed accidentally on day 21 from an unsuccessful attempt to bleed intracardially was negative by this test with both antigens. Titration tests with Melcher's antigen with R29 serum showed increasing antigen titer as the infection progressed. Serum taken 61 days after infection was positive with antigen diluted 1 : 8000 and negative with dilution 1 : 16000 (Fig. 3). All rat sera were negative by ring tests with Melcher's and saline antigens.

Fifty normal human sera, 19 schistosomiasis sera and 2 human trichinosis sera were tested with the precipitin ring test with Melcher's antigen. The human serum from the normal sample that was positive by hemagglutination was also strongly positive with the ring test. The two schistosomiasis sera positive by hemagglutination showed questionable positive ring tests with Melcher's antigen and negative ring test with cercarial and adult schistosome antigens. The remaining sera in each sample were negative.

Double diffusion tests in agar were made with one normal rabbit serum and 3 samples of serum obtained from two infected rabbits, 42 and 57 days after infection. No bands were observed in normal serum. Three bands were seen in infected serum.

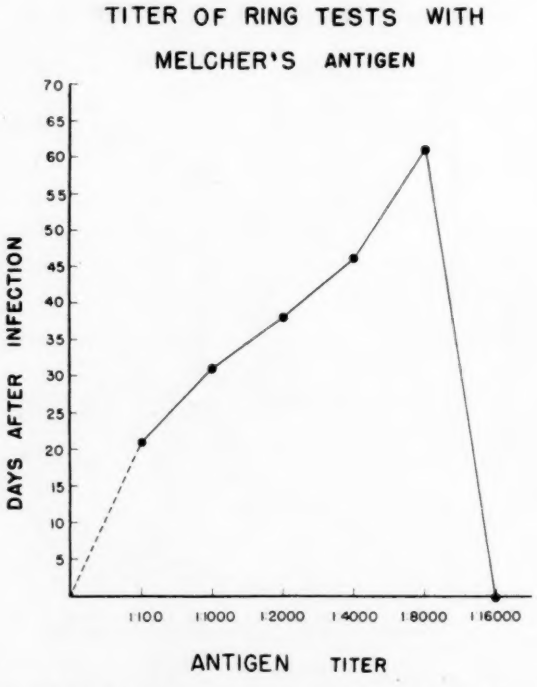

FIG. 3. Titer of Melcher's acid-soluble protein antigen in ring tests with the sera of rabbit experimentally infected with 12,000 larvae of *Trichinella spiralis*.

Bands one and three appeared in 24 hours and band two in 48 hours (Fig. 4). In two tests, after many days, a fourth band was seen but this was believed to be an artifact.

DISCUSSION

For detecting antibody early in infection in experimental trichinosis, the hemagglutination test compares favorably with the flocculation test. Thomas, *et al.*, (1953) reported positive titers with the flocculation test the eighth day after infection in 17 of 34 infected rabbits. These workers found that the titer of the flocculation test did not reflect the worm burden of the infected rabbits. With the hemag-

glutination test peak titers were not significantly different between individual rabbits.

Norman *et al.* (1955) and Sadun and Norman (1955) working with both Melcher's antigen and a saline extract of *Trichinella* larvae reported that Melcher's acid-soluble protein fraction was more sensitive and stable than a saline extract of larvae, with the flocculation test. We found that positive ring tests were detected earlier in experimental rabbits with Melcher's antigen than with a saline extract of larvae.

The number of components present in a given antigenic mixture can be resolved experimentally with various agar-serum techniques (Munoz, 1954). As shown

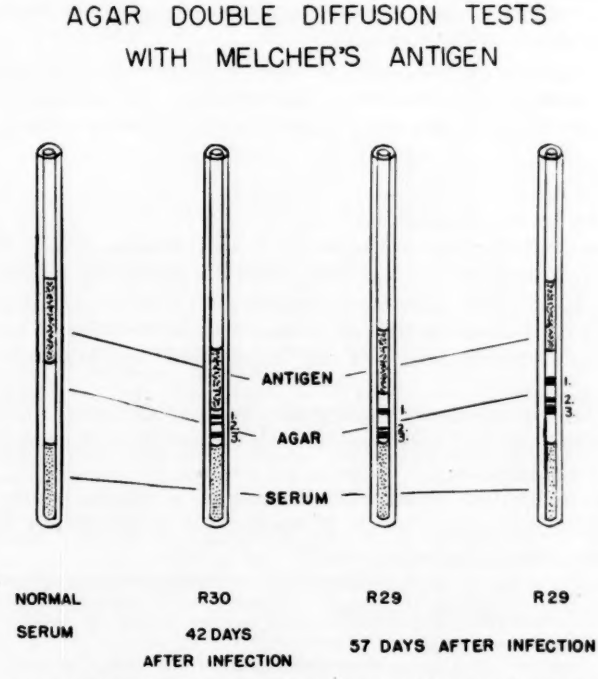

FIG. 4. Agar double diffusion tests with sera of rabbits experimentally infected with *Trichinella spiralis* and Melcher's acid-soluble protein larval antigen.

by Oudin (1952) only one precipitin band is formed when a single antigen-antibody system is present in a given mixture of antigen and antiserum. In systems showing multiple bands, these bands represent the minimum number of antigen-antibody systems, since one band may mask other bands in the same position. Melcher's antigen when tested against sera of infected rabbits shows a minimum of 3 antigen components. An acid-soluble trichina larvae antigen prepared without removing the lipid from the larvae has been analyzed electrophoretically by Portwood and Sanders (1946). These workers found 3 homogeneous peaks in their antigen. These peaks may correspond to the three antigenic components in Melcher's antigen.

Further work utilizing techniques which combine electrophoresis and agar diffusion (Williams and Graber, 1955) could probably demonstrate this relationship.

The fluctuations in hemagglutination titer with trichina antibody and cells coated with Melcher's antigen (Table I) were less marked than the tests made with schistosome antibody and cells coated with cercarial antigen (Kagan, 1955). The relative stability in the trichinosis system may be due to the fact that the acid-soluble larval protein fraction is a semi-purified, fractionated antigen, whereas the cercarial antigen used in the schistosome work was not fractionated. Antigens used by other workers in hemagglutination have been highly purified substances (Boyden, 1951, Stavitsky, 1954). Since Melcher's antigen is composed of at least 3 antigenic components perhaps if it were further fractionated with various concentrations of ammonium sulphate, the sensitivity and stability of the hemagglutination test could be further improved.

The hemagglutination test proved to be more sensitive than the precipitin test in detecting antibody in positive rabbit and rat sera. The hemagglutination test was also more sensitive than the precipitin test in detecting antibody early in infection.

SUMMARY

Rabbit and rat sera obtained after infection with *Trichinella spiralis* were tested for agglutinins with Boyden's hemagglutination test (Boyden, 1951). The antigen employed to coat sheep red cells was Melcher's acid-soluble larval antigen (Melcher, 1943). These same sera were tested with precipitin ring tests utilizing Melcher's antigen and a saline extract of trichina larvae. Agar double diffusion tests (Oakley and Fullthorpe, 1954) modified by Preer (unpublished) was made was Melcher's antigen.

Rabbits became positive by hemagglutination 6–15 days after infection. The hemagglutination test was more sensitive than the precipitin ring test in detecting antibody early in infection.

Melcher's antigen was superior to saline extracts of trichina larvae in the ring test since it detected antibody earlier in infection and in one instance was positive when the saline antigen was negative.

Agar double diffusion tests indicate that a minimum of three antigenic components are present in Melcher's antigen.

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RESEARCH NOTE

THE PROGENETIC TREMATODE *CERCARIA PATIALENSIS* SOPARKAR IN CEYLON

During a study of various cercariae and their snail hosts in freshwater habitats in Ceylon, the interesting progenetic and protandrous form *Cercaria patialensis* Soparkar, 1924, was found to infect the snail *Melanoides tuberculata* (Müller) in paddy-fields in Godapola, near Peradeniya. Of a total of 545 snails of this species, examined during February, April, September, October and December, 22 harbored this cercaria, while 228 had other types of cercaria. *Cercaria patialensis* seems therefore to occur throughout the year in this locality and to infect about 4% of the population of *Melanoides tuberculata*.

Study of living cercariae, of permanent, stained preparations of centrifugally concentrated material and of cercariae in stained, serial sections of snails, has confirmed most of the morphological details given by Soparkar (1924. Ind. J. Med. Res. **11:** 933) and Anantaraman (1948. Ind. J. Helminth. **1:** 11). The ovary, however, is in the position Soparkar described and figured it, i.e. on the left side, just anterior to the left testis. Anantaraman thought that Soparkar could not have seen an ovary in that position, and he himself described it as being "situated within the orbit of the acetabulum (as seen in an optical section) just behind the bifurcation of the oesophagus." I think Anantaraman was either mistaken on this point or describing some modification of this species. Study of the Ceylonese material by all three methods mentioned above has confirmed Soparkar's account of the genitalia.

This and another closely related form, *Cercaria koliensis* Olivier, 1947, will be viewed with special interest as further discoveries relating to their geographical distribution and life-histories are made. Soparkar's material came from *Melanoides tuberculata* from the Punjab, Northern India. Anantaraman's re-description was based on material from the same snail host in Buchireddipalayam near Nellore in Madras Presidency. Olivier (1947. J. Parasitol. **33:** 234) reported a similar form, *Cercaria koliensis*, from *Melanoides terebra* (?) in Guadalcanal, Solomon Islands, in the South Pacific.

The final hosts of these cercariae are still unknown. Anantaraman suggested that for *Cercaria patialensis* it might be a duck, but in an addendum to his paper he modified this view somewhat, since Olivier had suggested that *Cercaria koliensis* was related to the adult trematode, *Transversotrema haasi* Witenberg (1944. J. Parasitol. **30:** 179). The latter shows close morphological resemblance to both *Cercaria patialensis* and *C. koliensis* and came probably from the region of the gills or upper alimentary tract of some Red Sea fish. In view of the undecided nature of this problem, it is interesting to note that in the locality in Ceylon where *C. patialensis* has been found there are no ducks. There are only the Pond Heron, *Ardeola grayi*, and one or two kingfishers, among birds, but these have so far shown no signs of any infection with a trematode that could be regarded as the adult of this cercaria. We shall have to look probably to the freshwater fishes for an answer to this problem.—H. CRUSZ, *Department of Zoology, University of Ceylon, and Department of Parasitology, London School of Hygiene and Tropical Medicine, England.*

THE OCCURRENCE OF A DOG FILARIID OTHER THAN *DIROFILARIA IMMITIS* IN THE UNITED STATES

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INTRODUCTION

In the Fall of 1954, infected dogs were obtained from Norfolk, Virginia for studies on the mechanism of microfilarial periodicity. Reports to the contrary lacking, it had been assumed that microfilariae found in dogs in the United States were likely to be those of the heartworm, *Dirofilaria immitis*. The various studies on dog filariids in this country, including those on such subjects as incidence, intermediate hosts, treatment, relations between numbers of microfilariae in the blood and of adult female worms in the heart, appear to have assumed the existence of a single parasite. Thus, it was surprising to find that, of 8 infected dogs obtained from Norfolk, 3 had microfilariae which appeared to be different from those in the other 5. On the basis of Giemsa-stained smears of ear blood, one kind appeared to be thinner and more delicate than the other, tended to assume a tightly coiled position in contra-distinction to the latter's more usually sinuous appearance, and appeared to be more fragile in that the anterior end was often broken off. Also, although it was difficult to establish objective criteria in this regard, the smaller type seemed to be more active in fresh preparations than the other.

In the Spring of 1955, periodic checks of dogs purchased by the National Institutes of Health were initiated to provide a source of microfilariae. These dogs were obtained from suppliers covering the general areas of Pennsylvania, Maryland, and New Jersey, mainly from the former. Some 7% of over 250 dogs of all types have shown microfilariae on ear vein puncture, and, oddly enough, all but 3 of these have been of the "thinner variety.

Inasmuch as several species of dog filariids have been described in other parts of the world, Neveu-Lemaire (1936), Yorke and Maplestone (1926), Sprehn (1932), the possibility that the two apparent kinds of microfilariae were actually different, and did not represent some artifact, was investigated.

EXPERIMENTAL

Microfilarial size

Measurements were made with an ocular micrometer of microfilariae which had been subjected to the Knott technique and stained with methylene blue. One ml of ear blood was allowed to drip into 10 ml of 2% formalin, and this mixture was then shaken and centrifuged for 5 minutes at 1,500 r.p.m. The supernate was poured off, and the sediment was examined as a wet preparation after mixing with an equal volume of 1 : 1,000 methylene blue. After this treatment, most of the microfilariae were quite straight and clearly delineated.

Dog A was the first dog noted to be infected with the thicker type and, thus, the latter will be referred to as type A microfilariae; type F is the designation given

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** Laboratory of Tropical Diseases.

the thinner kind for the same reason. Length and width measurements were made on a total of 20 microfilariae from 2 dogs infected with each type. When prepared in this manner, the specimens of a type showed relatively little variation in dimensions, whereas between types there was a considerable difference. (Plate I, Figures 1 and 2.) Type A microfilariae were somewhat longer than the F type, averaging 313 (range: 307 to 322) microns in length versus 276 (range 269 to 283) microns, respectively. In the case of the width, there was a very marked difference between the two. Type A microfilariae averaged 6.9 (range: 6.7 to 7.1) microns in width, whereas type F averaged only 4.6 (range: 4.3 to 4.8) microns. The tail of the F type characteristically terminated, under these conditions of treatment, in a curved, buttonhook fashion. In contrast, the tail of the A type was straight. (Plate I, Figures 3 and 4).

Microfilarial internal anatomy

Comparisons were made of the locations, in relation to over-all length, of various anatomical structures and Anlagen in the two kinds of microfilariae, according to the method of Fülleborn (1924). These structures were not readily discernible in microfilariae treated with formalin prior to being stained with methylene blue or Giemsa. However, they were observed fairly readily in microfilariae in Giemsa-stained thick films examined under oil. The latter procedure was found to be quite satisfactory for locating all structures except the terminal nuclei. For determining the latter, the blood smears were stained by a hemalum technique (Feng, 1933). The linear distances of various anatomical loci from the anterior end were ascertained by means of an ocular micrometer. Only those microfilariae which were reasonably straight and in which all features, except the nuclei mentioned above, were recognizable in a single organism were included in the comparisons. Both kinds of microfilariae were handled similarly and simultaneously; so it was felt that any differences of significance could not be attributed to differential treatment.

In Table I there are presented the mean values obtained for the locations,

TABLE I.—*Mean location of various anatomical features in type A and type F dog microfilariae, expressed as percentage of length*

Locus	Mean percentage of length ± S. E.	
	Type A	Type F
Nerve ring	23.8 ± 0.2	20.8 ± 0.1
Excretory pore	32.7 ± 0.2	31.0 ± 0.1
Excretory cell	38.6 ± 0.2	34.5 ± 0.1
G_1 cell	67.9 ± 0.3	70.1 ± 0.3
G_2 cell	74.1 ± 0.3	75.7 ± 0.3
G_3 cell	75.4 ± 0.3	77.2 ± 0.4
G_4 cell	79.4 ± 0.3	78.6 ± 0.4
Anal pore	82.0 ± 0.2	80.7 ± 0.4
Last nucleus	92.7 ± 0.3	89.0 ± 0.4

antero-posteriorly, of various anatomical features in terms of the percentage of the total length. Each mean is based upon 10 microfilariae of each type. As is apparent from the standard errors, the location of a given feature was quite stable among samples of a given type.

Figure 5 is a schematic comparison of the two microfilariae in which the differences in location of the various anatomical details are more readily discernible. It would appear reasonable to conclude that the two microfilariae differ at least

in the relative location of the nerve ring, in the distance between excretory cell and G_1 cell, and in the distribution pattern of the G cells.

There appeared to be some difference in the tail nuclei, although the variation, especially among F type microfilariae, suggests that this feature may not provide a consistent basis for distinguishing the two. The A type terminated rather consistently in a pair of nuclei followed posteriorly usually by 5 individual nuclei be-

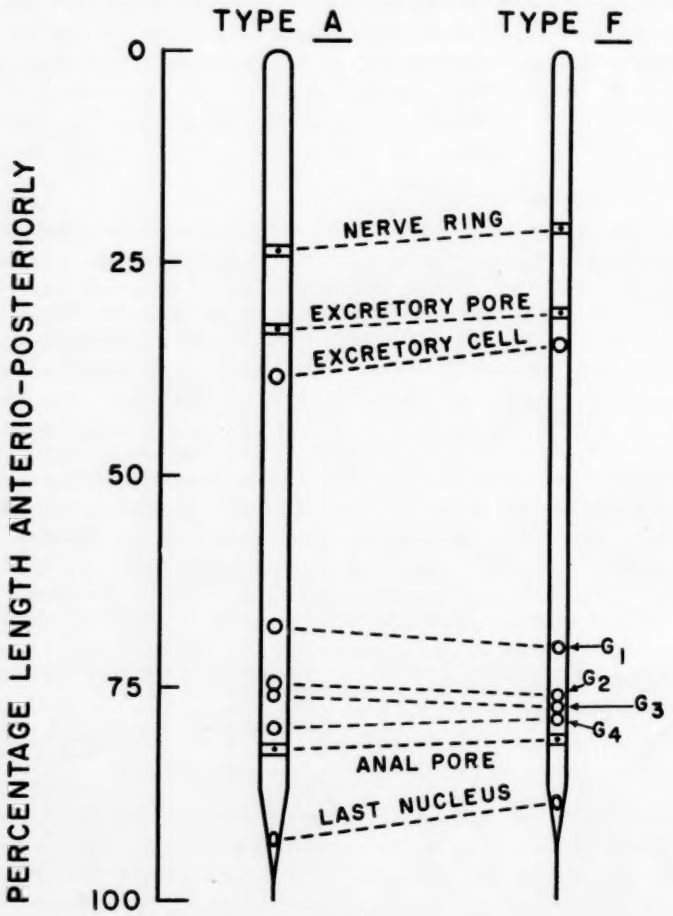

FIG. 5. Schematic representation of anatomical loci in type A and type F microfilariae.

coming narrower and more elongate. (Plate II, Figure 6.) With the F type there were often 4 to 7 individual nuclei some of which appeared to be fused and others fragmented. (Plate II, Figure 7.)

Microfilarial periodicity

At the time that a 24-hour observation period was undertaken to ascertain any fluctuation in microfilarial concentration, only one dog with a reasonable number

of the F type, Dog F, was available. As far as could be ascertained from repeated examination, this dog had only the one type. Of those with the A type, 2 suitable dogs (A and E) were available. These also did not appear to have mixed infections. All 3 dogs had been maintained side-by-side in cages under essentially the same rearing conditions for 8 months. Dogs A and E had been exposed for a few hours, on occasions, to certain experimental conditions which, as will be pointed out in a later report, did not appear to have any consistent effect upon the microfilarial concentration. However, inasmuch as Dog F did not receive such treatment, this fact is mentioned.

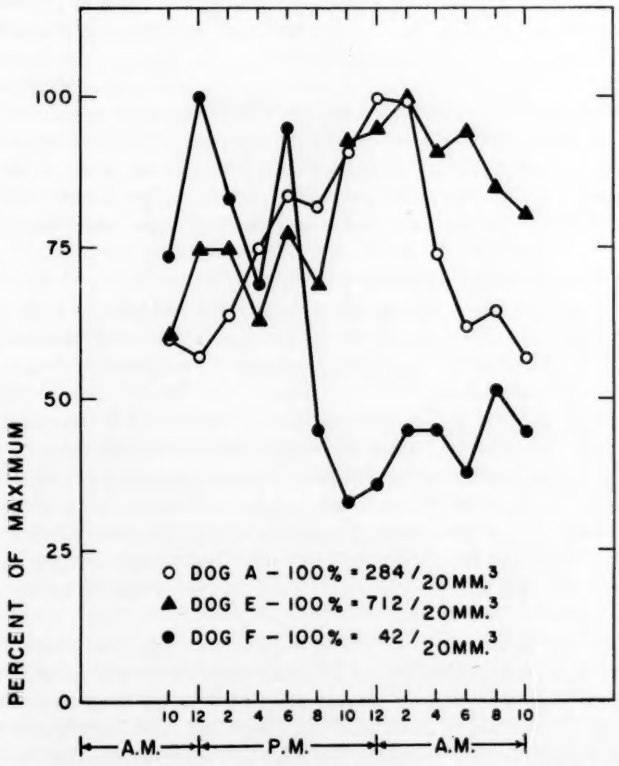

FIG. 8. Concentration of microfilariae in peripheral blood of two dogs with A type (A and E) and one with F type over 24-hour period.

Beginning at 10 a.m. on one morning and at 2-hour intervals thereafter until the same time the next morning, two 20-cu mm samples of blood (1 from each ear) were drawn by means of a hemoglobin pipette and prepared as thick smears about 15 to 20 mm in diameter on slides. The slides were stained with Giemsa and examined as wet preparations. All microfilariae were counted and the average of the numbers on the 2 slides at a particular period was considered as indicative of the concentration in the blood at that time.

In Figure 8, the results of the 24-hour study of the 3 dogs are expressed graph-

ically as curves. Each point on a curve is the percentage, at a particular time, of the maximum microfilarial density throughout the 24 hours. These maxima for dogs A, E, and F were 284, 712, and 42 microfilariae per 20 cu mm of blood, respectively.

The considerable fluctuation is apparent. Only in Dog A did the microfilariae exhibit a relatively smooth cycle; in neither E nor F did the counts return to the original values in 24 hours. While there was evidence of a periodicity, it did not appear to be very marked. With the A type (Dogs A and E) there was less than a two-fold difference between maximum and minimum counts, although this difference appeared to be greater in the case of the F type. However, there was considerable difference between the types in the time at which the microfilarial peak occurred. In the case of the larger microfilariae, the highest concentrations in the peripheral blood occurred late at night to early in the morning. On the other hand, the type F microfilariae tended to be at their lowest concentration in the peripheral blood at this time. Thus, there was an approximate 12-hour difference between the 2 kinds in corresponding phases of microfilarial concentration, although the failure to return to starting values in some instances, suggests that these cycles are not necessarily clearcut, certainly in the case of the F type microfilariae.

Development in possible intermediate hosts

While studies on the comparative abilities of the two kinds of microfilariae to undergo normal further development in arthropods are still underway, certain observations thus far furnish additional evidence to support the belief that more than one parasite is present.

From 4 dogs with the F type microfilariae, 71 fleas, in total, have been recovered and dissected. Seventeen of these contained fully developed infective-type third stage filariid larvae, and many others had larvae in earlier stages of development. They appeared to lie in the hemocoelomic spaces, and none was found in the Malpighian tubules. Some contained as many as 10 to 15 active third stage larvae. Positives and negatives were found among both *Ctenocephalides canis* and *C. felis*, and among both male and female fleas. Each of the 4 dogs contained fleas with third stage larvae.

Relatively few fleas which had fed on dogs with A type microfilariae have been available for dissection. Thus far, of 23 which were either recovered initially from a dog with A type infection, or were recovered 7 to 10 days after having been placed on the latter from an uninfected dog, none has contained developing larvae. Some fleas contained many parasites, but they had not progressed beyond the early first or microfilarial stage and many were degenerate forms of this stage. It would be well to examine more fleas from dogs with A type microfilariae to be certain of this apparent inability of the latter to develop in dog fleas. However, it is quite certain that the F type parasite will develop normally in fleas, and it seems that the two types differ in this regard.

With regard to development in *Anopheles quadrimaculatus*, the two types differ quite markedly. Of 55 mosquitoes which survived 10 to 14 days after feeding on 2 dogs with A type microfilariae, 48 contained second and third stage, and infective larvae. Some contained as many as 40 such larvae, several of which were in the head and proboscis. Development took place in the Malpighian tubules.

In contrast, of 117 mosquitoes which survived the same period after feeding on 3 dogs with F type microfilariae, none was positive. (Incidentally 2 of these 3 dogs had harbored most of the fleas which had contained infective larvae.) Because dogs with similar concentrations of the 2 kinds of microfilariae were not available, there was a considerable difference in uptake by the mosquitoes. Dissections made immediately after feeding reflected this difference, but also demonstrated that the negative results obtained with mosquitoes fed on dogs with the F type could not have been due to the failure to ingest microfilariae. As many as 8 to 11 microfilariae were often recovered immediately after feeding. Thus, it is quite certain that the A type microfilariae undergo normal development in *A. quadrimaculatus,* whereas it appears that the F type do not.

Recovery of adults

Four dogs with A type microfilariae (including dog A) were autopsied and examined for adult filariid worms. Three had high microfilarial counts; the concentration in the other was 30 to 40 per 20 cu mm at 2:00 p.m. Male and female filariids which were indistinguishable from *Dirofilaria immitis* were recovered from the right ventricle and pulmonary artery (mostly from the latter) in the 3 heavier infections. Other parts of the dogs were not examined, inasmuch as only a single kind of microfilaria was observed in each of the dogs and it was felt that the microfilariae were probably derived from the heartworms.

The results obtained with Dog A corroborated this belief. This dog was found to contain one female and one male in the right ventricle and pulmonary artery. (The finding of only a single pair of worms is of interest inasmuch as the microfilarial count on this dog averaged more than 200 per 20 cu mm in the afternoon at the time of autopsy, whereas counts a year earlier had averaged about half this figure.) The female was removed and washed in Ringer-glucose solution several times to remove the blood and microfilariae adhering to its surface. The worm was then placed in fresh solution, the latter examined to make certain there were practically no microfilariae present, and the container then placed in a 37° C incubator. In a few hours, there were numerous microfilariae which had been liberated by the female in the container. These were centrifuged, formalinized, and again centrifuged, and examined after being stained with methylene blue. Measurements were made on these microfilariae known to be derived from *Dirofilaria immitis* and they were found to agree quite closely with those of the microfilariae found in the peripheral circulation of this dog: 306 (range, 298 to 312) by 6.6 (range, 6.4 to 6.9) microns. Thus, it is beyond question that the A type microfilariae are those of *D. immitis.*

Five dogs with the F type microfilariae (including Dog F) have been examined for adults. One (Dog L) had a count of 25 microfilariae per 20 cu mm at 2:00 p.m., the others had counts considerably below this at the time of autopsy. In no instance was an adult worm found. Hearts and lungs were examined in all instances. In the case of 3 dogs, the subcutaneous and peritoneal and pleural areas were also examined. In the case of 2 of these (Dogs F and L), practically the entire dog, including fat, skin, organs, and muscles, was washed and soaked in warm saline and carefully teased apart in search of adult filariids.

The failure to find adult worms in the heart in these dogs does not in itself, of course, preclude the possibility of heartworm. Such results can be expected, par-

ticularly where such low microfilarial concentrations are present. On the other hand, if the F type microfilariae arise from some tissue-inhabiting filariid, as is our opinion at present, one might also anticipate difficulty in locating adults in the case of such low microfilaremias. Dogs are continuously being screened for heavier F type microfilarial infections with the hope of increasing the chances for finding the corresponding adults somewhere in the body.

DISCUSSION

Insofar as can be ascertained from the literature, and from discussions with others who have worked on or are interested in filariasis, this is the first demonstration that a dog filariid other than *D. immitis* occurs in this country. Although others may have felt that such must exist, we can find only one description of an attempt to investigate this question, Mudhenk and Greene (1939). They suggested that there may be another parasite to explain the fact that they so often failed to find heartworms in dogs with blood parasites. They also felt they had an "haematozoan" which was different from microfilariae of *D. immitis*, although little descriptive material is provided. They suggested the possibility that it was a larval stage of *Spirocerca sanguinolenta* which was present in a high percentage of the dogs they examined. Also, on the possibility that it was a filariid that inhabited the subcutaneous tissues, they examined "a large number of dog carcasses" without success.

Failure to find adult filariids in the hearts of dogs with microfilariae appears to have been a fairly common experience. Brown (1939) in North Carolina, in a survey of *D. immitis* in dogs, noted that in several instances microfilariae were found in the absence of mature worms in the heart or lungs. In fact, of 63 dogs found either by blood examination, or heart and lung examination, to be infected, 18 showed microfilariae without adults in the latter sites. Eyles, *et al.* (1954) in a *D. immitis* survey in Memphis, Tennessee found in a series of 204 dogs on which examinations of both blood and heart and pulmonary vessels were made, that only a little more than 20% of those found positive for microfilariae revealed adults in the heart. The likelihood that microfilariae can persist in the circulation after the death of the adult heartworm and the possibility that a light infection with the latter can be missed might explain some of these findings. However, the existence of another filariid located elsewhere in the host could also, of course, contribute to the same results.

There appears to be some conflict of opinion as to the suitability of dog and cat fleas as hosts for *D. immitis*. One must always take into account, of course, the possibility that strain differences in both parasite and arthropod, and differences in microfilarial densities might be at the basis of some of the discrepancies. However, in other instances, such explanations cannot resolve the uncertainty. Among the several reports that *D. immitis* develops successfully in fleas is that of Breinl (1921) in Australia who found developing and mature larvae in fleas collected from dogs. However, although it is stated that *D. immitis* was common in the area, nothing is mentioned about the adult present in the hosts used other than that "fleas were collected from dogs known to harbor the parasite in their peripheral blood." Heartworm may have been present in the dogs, but no evidence is presented to rule out the possibility of the existence of another microfilaria. Brown and Sheldon (1940) in North Carolina also found development to the infective stage in fleas

collected from a dog considered to have heartworm because it had microfilariae. However, no evidence is presented that the microfilariae in this dog were those of, or limited to, *D. immitis*. Interesting, also, was the fact that the same authors stated that they "have examined numerous fleas from dogs heavily infected with *Dirofilaria* microfilariae and have failed to detect developmental stages of this parasite." Summers in Louisiana (1940, 1943) also found excellent development in fleas, collected from or fed on microfilaria-infected dogs. However, here too, there is no direct evidence provided that the particular dogs used had heartworms, or heartworms only. Steuben (1954), in intensive studies of the larval development of *D. immitis* in fleas, found more than one-third of the many fleas collected from 71 dogs to contain various developmental stages of filariid larvae. He also dissected many fleas experimentally fed on an infected dog with similar results. However, autopsies of dogs were not mentioned and while it is to be expected that *D. immitis* was present, if not the prevalent parasite, it is possible in the light of the information we have obtained that the infection in the fleas might have been that of some other filariid.

In the school of those who found that fleas were not suitable hosts for *D. immitis* there are some who undertook to identify the adult parasites, although this has not been the rule. Grassi (1888), in Italy, examined fleas from dogs which were demonstrated on autopsy to contain heartworms. Among several hundred fleas examined, he found only dead "Haematozoen" in the gut. In Germany, Kosuge (1924) was unable to find parasites in fleas from dogs "die vor mehreren Jahren experimentell mit *Dirofilaria immitis* infiziert worden waren und reichlich Mikrofilarien in ihrem Blut beherbergten." In France, Joyeux and Sautet (1938), found 2% of the fleas of microfilaria-harboring dogs infected with the parasite. However, none had attained the sausage stage, and they eventually degenerated. They were called *D. immitis*, although no further evidence was provided. Phillips (1939), in Massachusetts, examined 21 fleas taken from a dog heavily infected with microfilariae. None was infected but it is not known whether adults of *D. immitis* were recovered from this dog. Taniguchi, *et al.* (1944) examined 55 *C. canis* recovered from a dog shown at autopsy to contain *D. immitis* in the heart, with negative results. Rosen (1954), in French Oceania, was unable to find development further than the first stage in fleas collected from microfilaria-infected dogs. There was no question in this case, inasmuch as the dogs were shown at autopsy to harbor *D. immitis*. Because of the conflicting reports on the ability of *D. immitis* to develop in fleas, Rosen posed the question as to whether another filariid (perhaps *Dipetalonema reconditum*) might not have been involved in certain cases where development was obtained.

It is of interest that all the dogs with F type infection that we have been able to study have had very low microfilarial densities in the peripheral blood. On the other hand, most of the heartworm-infected dogs have had rather high counts. It is to be wondered whether low microfilarial densities in the peripheral circulation are characteristic of the F type infection, for reasons as yet undetermined. As indicated earlier, Brown and Sheldon (1940) could not find developmental stages in numerous fleas collected from dogs with heavy infections of microfilariae, whereas the dog from which infected fleas were recovered had an extremely low count. It seems more difficult to accept the possible explanation offered, i.e., that the absence

of infection in fleas recovered from heavily infected dogs is due to the death of the fleas because of the infection, than to consider the possibility that the parasite in the lightly infected dog may have been the F type.

Certainly, in retrospect, one can find in the literature sufficient basis to suspect that there may be more than one dog filariid in this country. Our findings extend this suspicion toward a conclusion. The finding of two kinds of microfilariae with such differences in size, internal anatomy, perhaps periodicity pattern, and differential development in two kinds of arthropods warrants such a conclusion. The A type microfilariae were demonstrated to be *D. immitis* because they were recovered from adults of the latter. Their size is within the ranges reported for this species. The locations of morphological characters do not agree too well with those reported in Fülleborn (1924). However, it is felt that such comparisons are best made when it is certain that the microfilariae under study have been treated similarly. Although our observations are limited in this respect, the failure to develop in fleas is consistent with results obtained by others who demonstrated that they were dealing with *D. immitis* by recovering the adult. The ability to develop in *Anopheles quadrimaculatus* is consistent with the results Phillips (1939) obtained after feeding this species on the same dog from which negative fleas had been recovered. As indicated earlier, he felt that he was dealing with *D. immitis,* although there is no autopsy evidence provided.

Thus far, we have not been able to find any adult filariid in dogs with F type microfilariae to help identify this parasite. Among the species mentioned by Neveu-Lemaire (1936), the one that best fits a microfilaria of such narrow width is *Dipetalonema reconditum* (Grassi, 1890). The latter is stated to have a width of 4.3 microns, and our mean width figure for the F type microfilaria was 4.6 microns, although without the certainty that the microfilariae were treated similarly, such comparisons must be made with caution. Also, our results on the ability of this type to develop in fleas were similar to those of Grassi and Calandruccio (1890) who studied the development of what they called *"Filaria recondita"* in fleas. However, there appears to be very little information on the adult of this parasite, which is said to occur in the subcutaneous connective tissues and peritoneal fat.

We are continuing dog surveys to find heavier F type infections. In the areas from which the dogs we examined are drawn, this appears to be the more prevalent of the two kinds. However, the infections have almost invariably been light, if one can use microfilarial counts in peripheral ear blood as a true measure of adult burden, and this may account for the inability to find adults thus far. Also, we are undertaking to transmit both types of infection in the laboratory in an effort to obtain adults, particularly those of the F type. Until, of course, we can study the latter and its location, we can only speculate that it is a tissue filariid, and perhaps is *D. reconditum.* It is felt, nevertheless, that this demonstration of another microfilaria in the peripheral blood of dogs in this country may explain some of the apparent inconsistencies in the literature on *Dirofilaria immitis.* Perhaps some of the dogs thought to harbor heartworms because of the occurrence of microfilariae in the peripheral circulation actually had the other parasite, exclusively or in addition to the former. This demonstration should be of considerable interest to the veterinarian as well as the parasitologist.

SUMMARY

Microfilariae which are different from those of *Dirofilaria immitis* have been found in peripheral blood of dogs obtained from areas in Virginia, Maryland, Pennsylvania, and New Jersey. Comparison with *D. immitis* microfilariae after treatment with formalin and methylene blue indicates that they are shorter than the latter, 276 microns vs. 313 microns, and are considerably narrower, 4.6 vs. 6.9 microns. The locations of various anatomical structures such as nerve ring, excretory cell, G cells, etc., differ between the two microfilariae in Giemsa-stained thick films. The unknown species develops to infective type third stage larvae in the body cavity of *Ctenocephalides canis* and *C. felis,* but fails to develop in *Anopheles quadrimaculatus.* In the present study, the reverse was found for microfilariae of dogs shown at autopsy to contain *D. immitis.*

Thus far, only light infections with this microfilaria have been encountered, which may condition the fact that no adult has been recovered from heart, or subcutaneous and peritoneal areas. The size and its development in fleas suggest that this microfilaria may be that of *Dipetalonema reconditum* which is said to be a tissue filariid. Little information is available on the latter, and until an adult is recovered in these infections identification of the parasite can only be speculative.

This is apparently the first demonstration that more than one dog filariid occurs in this country. This finding may shed light on some of the apparent discrepancies and unexplained findings reported for the dog heartworm.

ADDENDUM

Since the preparation of this manuscript, 5 adult filariids (1 male and 4 females) were recovered from the subcutaneous tissues of a large female hound with the F type microfilariae. No worms were found in the heart or pulmonary vessels. The microfilarial count on this dog averaged over 200 per 20 cu mm of peripheral ear blood at 10 a.m. Only a single kind of microfilaria was observed, and a couple liberated into the fixing fluid by the females were similar to those occurring in the blood. The adult worms are relatively small; the female is about 32 mm in length and 182 microns in width. The male, which had several caudal loops, measured approximately 17 mm in length and 106 microns in width. Preliminary observations indicate that the parasite is a species of *Dipetalonema.* A complete description will be published later.

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EXPLANATION OF FIGURES

PLATE I

FIG. 1. A type microfilaria after treatment with formalin and methylene blue. (×333)

FIG. 2. F type microfilaria after treatment with formalin and methylene blue. (×333)

FIG. 3. Caudal end of A type microfilaria after treatment with formalin and methylene blue. (×1176)

FIG. 4. Caudal end of F type microfilaria after treatment with formalin and methylene blue. (×1176)

PLATE II

FIG. 6. Terminal nuclei in A type microfilaria (×1225).

FIG. 7. Terminal nuclei in F type microfilaria (×1225).

PLATE I

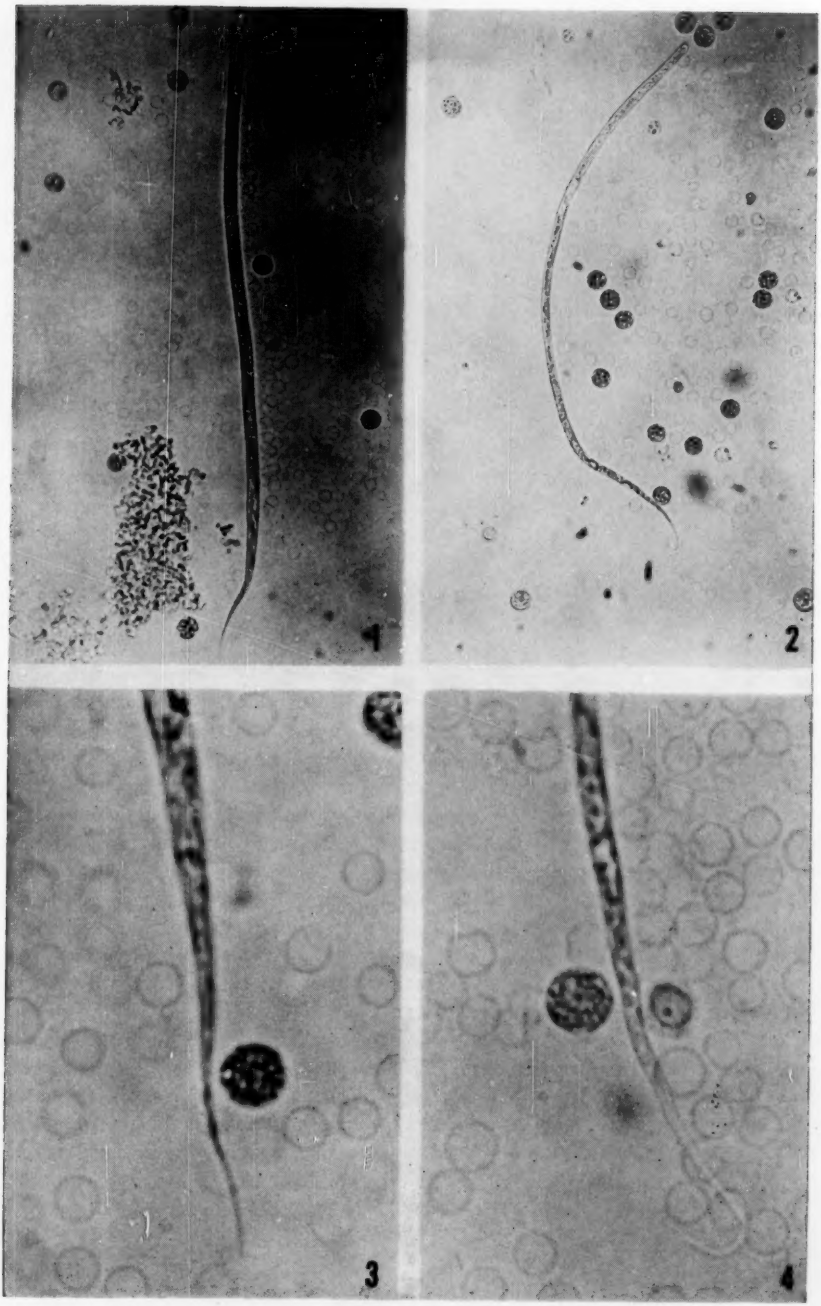
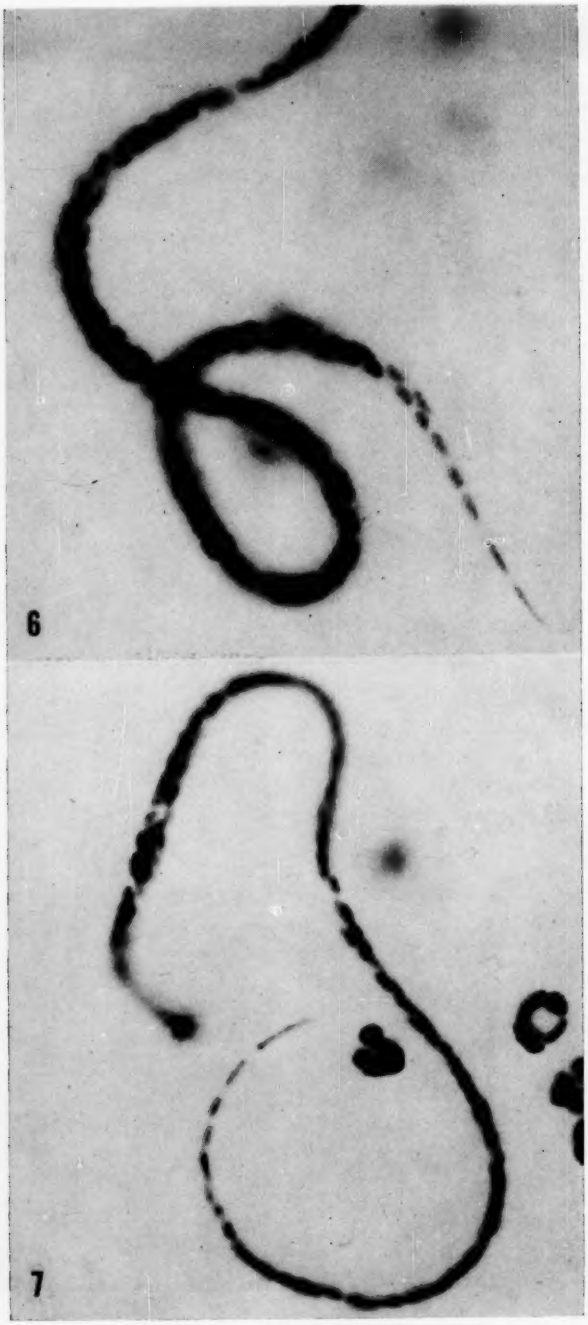


PLATE II



STUDIES ON THE HELMINTH FAUNA OF ALASKA. XXVII. THE OCCURRENCE OF LARVAE OF *TRICHINELLA SPIRALIS* IN ALASKAN MAMMALS

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In 1948 much interest in trichinosis in arctic regions was aroused, particularly by the findings of Thorborg *et al.* (1948), who investigated serious outbreaks occurring among the Eskimo of West Greenland during 1947. Consequently, with the founding of the Arctic Health Research Center in the autumn of 1948, a study of trichinosis in Alaska was the first project to be initiated by the Zoonotic Disease Section (formerly Animal-borne Disease Section) of this Center.

Field work was begun in January, 1949, and a preliminary note on trichinosis in Alaskan mammals was published by Brandly and Rausch (1950). The subject of trichinosis in arctic regions was reviewed by Connell (1949).

The survey to determine the prevalence of *T. spiralis* in mammals in Alaska was terminated in the spring of 1953; this paper reports the results of this work.

MATERIALS AND METHODS

An effort was made to obtain a series of each species of mammal occurring in Alaska, with exception of the ungulates. Such specimens were collected over the entire Territory, but most originated above the Arctic Circle. Many mammals were secured through the cooperation of Eskimo and Indian trappers, who held the frozen carcasses of animals taken for their fur until they could be brought to the laboratory in Anchorage. In some instances, only muscle tissue of animals, with collecting data, was preserved. The personnel of the Zoonotic Disease Section also collected large numbers of mammals. Dogs were generally obtained when those no longer serviceable were destroyed, but some were purchased for this study. Although some autopsies were performed in the field, most of the animals were brought entire to the laboratory. For the purposes of this work, several grams of striated muscle were taken, routinely from the diaphragm, at the autopsy of each animal. These samples were frozen and stored for later processing. The larvae were usually dead when isolated.

The presence of *Trichinella* larvae was determined through the artificial digestion of tissue samples. For this purpose, the tissue was chopped with a knife into small pieces, placed in a solution of pepsin and hydrochloric acid, and incubated at 37° C. with constant agitation for 24 hours. The resulting fluid was poured through a fine-mesh screen to remove any undigested material and was allowed to stand in graduated cylinders until any larvae present settled out. The supernatant fluid was siphoned off, the residue placed in watch glasses with a few drops of 10 percent

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potassium hydroxide solution, and any larvae present were counted under the dissecting microscope.

The tissue samples were processed at the Arctic Health Research Center during the summer of 1949 by Dr. J. D. Tiner, now at the Microbiological Institute, Rutgers University. From the spring of 1950 to the time the survey was discontinued, this work was performed by Mr. B. B. Babero. This report is based upon the data recorded by these workers.

RESULTS

Tissue samples from 2433 mammals, representing 42 species, were processed. Larvae of *Trichinella spiralis* were found in 23 species. Two hundred eighty-five animals (11.7 percent of the total) harbored larvae.

The findings are discussed below, by host-species. Mammalian names are according to Miller and Kellogg (1955) and Rausch (1953).

Soricidae.

No larvae were found in 18 shrews of 3 species; viz., *Sorex obscurus* Merriam, *S. cinereus* Kerr, and *S. tundrensis* Merriam. Shrews feed readily upon carrion, and in Alaska they are found often in buildings or caches where meat is stored.

Beliaeva (1954) examined a total of 126 shrews from the Belovezha Forest, on the eastern border of Poland. One of 39 specimens of *S. araneus* Linnaeus, and one of 38 specimens of *S. macropygmaeus* Miller (= *S. caecutiens* Laxmann) were infected. Forty-nine specimens of *S. minutus* Linnaeus were negative.[3] Beliaeva concluded that these animals became infected through feeding on carcasses of carnivorous mammals.

Ursidae.

Three species of bears occur in Alaska, and small series of each were obtained.

Of 20 grizzly (brown) bears, *Ursus arctos* Linnaeus, 10 (50 percent) harbored *Trichinella* larvae. These ranged in number from 0.1 to 45.6 larvae per gram of tissue (av. 12.4 per gram). The bears were from localities scattered widely over the Territory, and infected animals were taken as far north as the Alaktak River, near the Arctic Coast, and as far south as the lower Alaska Peninsula.

These bears feed upon carrion whenever it is available, including carcasses of other bears killed and left in the field by hunters. They are mainly herbivorous, however. Farther south (Kodiak Island and Alaska Peninsula), salmon (*Oncorhynchus* spp.) comprise an important part of the summer diet.

Five black bears, *Ursus americanus* Pallas, of 23 examined, were infected (21.7 percent). Numbers of larvae ranged from 0.5 to 400 per gram of tissue (av. 81 per gram). Most of the bears examined, and all of those infected, were killed in southern Alaska. Black bears feed largely upon vegetation, but also consume available carrion. They are numerous near settlements and farms, where they sometimes feed upon refuse.

Nine of 17 (52.9 percent) polar bears, *Thalarctos maritimus* Phipps, harbored *Trichinella* larvae. Infections were very light, ranging from 0.4 to only 4.0 larvae per gram of tissue (av. 1.6 per gram). Excepting 3 animals from St. Lawrence

[3] Beliaeva gave only common names for mammals examined; the scientific names of the insectivores were obtained from Bobrinskii, B., A. Kuznetsov, and A. P. Kuziakin, "Opredelitel' mlekopitaiushchikh SSSR." Moscow, 1944.

Island, all were killed on the Arctic Coast near Barrow or Wainwright.

Leiper (1938) reported 4 of 6 polar bears dying in the London Zoological Garden to be infected with *Trichinella* larvae, and he believed these to have been acquired before the animals were captured. There have been several reports of infected bears from zoological gardens on the Eurasian continent. Parnell (1934) reported having found infected polar bears in the Canadian Arctic. Thorborg *et al.* (1948) observed larvae in 2 of 3 polar bears from the Thule region, and in 4 of 13 from East Greenland. Roth (1949) found larvae in 6 of 19 bears from West Greenland. Brown *et al.* (1949) observed larvae in 2 of 3 bears from the Canadian Northwest Territories. Roth (1950) summarized the work in Greenland, and reported infections in 31 of a total of 112 polar bears examined. He also reviewed the unpublished data of other workers, and mentioned the finding of infected bears in Spitzbergen.

Outbreaks of trichinosis in man have been traced frequently to their origin in the flesh of polar bears (see Connell, 1949; Roth, 1950).

In contrast with the aforementioned species, the polar bear is almost entirely carnivorous, although some vegetation may be consumed during the summer months. Seals (*Phoca* spp.) are an important part of its diet. Polar bears sometimes enter Arctic Coast villages, where they feed on carrion, including carcasses of dogs and other animals.

Canidae.

A total of 225 dogs was examined from various localities. Of these, 102 animals (45.3 percent) harbored *Trichinella* larvae. These figures, however, are misleading, since place of origin is important. Sixty dogs, all negative, were obtained in Anchorage and its environs—the one city in Alaska most nearly comparable to small cities in the United States. Excluding these, 61.8 percent of the remaining 165 dogs were infected.

More specifically, 40 of 47 dogs (85.1 percent) from St. Lawrence Island harbored larvae, and 38 of 41 dogs (92.6 percent) from the village of Barrow were infected. In the dogs of these 2 villages, the number of larvae per gram ranged from 0.5 to 65, with an average of 9.7 per gram.

Included in this series of 225 dogs were 13 from the Aleutian Island of Adak; of these one animal was infected, having 4 larvae per gram of tissue. Rats, *Rattus norvegicus* Berkenhout, are extremely abundant on Adak, and are frequently infected (12 percent of 224 rats; Schiller, 1952). The dogs examined were strays, but it appears that they did not often feed upon rats. Sixty-four dogs not mentioned specifically, were collected from numerous localities, mostly in northern Alaska.

It is evident that conditions in arctic villages favor the infection of sledge dogs. Although only a few species of land mammals occur on St. Lawrence Island, the arctic fox is trapped there annually in large numbers (the population has been high during the last 10 years) and the discarded carcasses of these animals are often consumed by dogs. The dogs are also fed waste parts of those marine mammals that provide the main source of food for the Eskimo. Conditions are somewhat comparable at Barrow, where foxes were abundant in 1949–50, and again in 1953–54. At both places, discarded carcasses of dogs are eaten by other dogs,

especially in the summer, when the animals are not worked and are consequently fed little.

From previous work in arctic regions it is known that the prevalence of *Trichinella* larvae in dogs may be high. Thorborg *et al.* (1948), in an addendum, reported 41 of 54 dogs from West Greenland infected. According to Roth (1949), 46 of 66 dogs from the same region were infected; in a later report (1950) summarizing all of the work in Greenland, larvae were reported in 151 (66.5 percent) of 227 dogs examined. Kuitunen-Ekbaum and Fleming (1949) found 2 of 4 dogs infected in northern Canada.

Of 8 coyotes, *Canis latrans* Say, examined from southern Alaska, one (12.5 percent) had 45.0 larvae per gram of tissue. Since coyotes feed essentially upon small mammals and carrion, examination of a large series would probably disclose a high prevalence of infection.

Tissues were processed from 154 wolves, *Canis lupus* Linnaeus, most of which were killed by Eskimo in the central Brooks Range, arctic Alaska. Fifty-one wolves (33.1 percent) harbored larvae. These ranged in number from 0.1 to 20 per gram of tissue (av. 3.3 per gram).

From these findings, one might conclude that wolves feed upon smaller mammals to an extent greater than ordinarily supposed. Certainly they rarely, if ever, become infected through the eating of ungulates. According to the observations of one of us (RR), little carrion besides that of large hoofed animals is available in the nearly uninhabited region of interior Alaska where most of these wolves were taken.

Roth (1950) demonstrated *Trichinella* larvae in fragments of tissue adhering to skins of 2 wolves taken in Greenland and deposited in the Zoological Museum of Copenhagen. In the Belovezha Forest, Beliaeva (1954) found larvae in 8 of 21 wolves examined.

In Alaska, the red fox, *Vulpes vulpes alascensis* Merriam, is also an important host of *T. spiralis*. Of 76 animals examined, 31 (40.8 percent) were infected. The number of larvae ranged from 0.3 to 41 per gram (av. 8.4 per gram). Fifty-four of the red foxes (71 percent of the total) were collected in the Brooks Range, and the remainder from various localities to the south. Of these 54 foxes, 25 (80.6 percent of the total number of infected animals) harbored larvae. Forty-four foxes were taken around Anaktuvuk Pass, central Brooks Range; of these, 20 (45.5 percent) were infected. Farther to the east in the vicinity of Arctic Village, 10 foxes were taken, 5 (50 percent) containing larvae. Although the series are small, it is of interest to compare with the above the findings in 13 foxes killed in the Copper River valley south of the Alaska Range; here, only 2 animals (15.4 percent) harbored larvae. The remaining 9 infected red foxes were taken from widely separated localities.

To our knowledge, nothing has been published previously on the occurrence of *T. spiralis* in the red fox in arctic America. This species is known to be an important host of this nematode in Europe. Roth (1950) summarized data on *Trichinella* larvae in the red fox there, and additional work has been done by Söntgen (1939) and by Beliaeva (1954), who found larvae in 5 of 6 red foxes from the Belovezha Forest, in eastern Poland.

Only 16 (7.2 percent) of 222 arctic foxes, *Alopex lagopus* Linnaeus, were in-

fected. Larvae ranged in number from 0.1 to 84.5 per gram (av. 22 per gram). Of 117 foxes from the Arctic Coast, 12 (10.3 percent) were infected. Of 94 arctic foxes from St. Lawrence Island, 3 (3.1 percent) were infected. The other infected fox was collected on Adak Island, in the Aleutians.

Parnell (1934) observed infected arctic foxes in northern Canada. Leiper (1938) reported trichinosis in 2 arctic foxes which died in the London Zoological Gardens. Roth (1949) found 3 of 101 foxes from West Greenland infected (2.8 percent), and in a later report (1950) had found no more after examining 264 animals. In the latter paper, Roth reviewed other work done in Greenland and elsewhere.

On the Arctic Coast and St. Lawrence Island, the arctic fox is dependent essentially upon mouse-like rodents for food. The availability of food varies according to population fluctuations among the small rodents. During the last 10 years, the brown lemming reached high population densities 3 different times on the Arctic Coast. The tundra vole on St. Lawrence Island has been abundant since 1949, attaining greatest numbers in fall, 1954. Other food items are invertebrates cast in by the sea, young birds, and carrion (see Braestrup, 1941). Remains of marine mammals are consumed, including those of seals left by polar bears.

Mustelidae.

A high prevalence of *T. spiralis* larvae was recorded in the ermine, *Mustela erminea arctica* (Merriam). Of 51 animals examined, 18 (35.3 percent) were infected. The larvae ranged in number from 0.1 to 436 per gram of tissue (av. 16.4 per gram). Of 40 animals collected in the Brooks Range, 17 (42.5 percent) were infected. The remaining ermines, including the other infected animal, were collected in the Copper River valley. Roth (1950) examined 3 ermines with negative results.

Since the ermine feeds upon mouse-like rodents, the high prevalence of *T. spiralis* is not explained by present knowledge. Mouse-like rodents and hares may harbor *Trichinella* larvae more frequently than recognized. The large number of small mammals consumed by the ermine and other small carnivores might compensate for a low rate of infection. This principle holds true for certain other species of helminths in Alaska; i.e., the larval form may seem very rare, but a high proportion of the final hosts may harbor the adult.

Two least weasels, *Mustela rixosa eskimo* (Stone), harbored larvae, one having only 0.8 larvae per gram of tissue, and the other more than 1400 per gram. Both were collected in the central Brooks Range. These small weasels prey upon lemmings and other small rodents, and may become numerous when the prey-species reach a high density.

Nineteen of 38 wolverines (*Gulo gulo* Linnaeus) (50 percent) studied contained larvae of *T. spiralis*. The number of larvae ranged from 0.2 to 18 per gram of tissue (av. 3.5 per gram). Rates of infection in this series of wolverines did not differ with the locality from which they were taken.

In addition to feeding upon small mammals, the wolverine consumes carrion.

The following mustelids were examined with negative results: Marten, *Martes americana actuosa* (Osgood), 18; Mink, *Mustela vison* Schreber, 43; Otter, *Lutra canadensis* (Schreber), 7; Sea otter, *Enhydra lutris* (Linnaeus), 20. An exami-

nation of larger series of mink and marten is planned. Many mink are trapped by the Eskimo of the lower Kuskokwim region, and the carcasses of these animals are eaten during the winter months.

Felidae.

Four of 17 lynx, *Felis lynx canadensis* Kerr, harbored larvae (23.5 percent), ranging from 0.6 to 14 larvae per gram of tissue (av. 7.2 per gram). Two of the infected animals were taken in the central Brooks Range, and 2 in the Copper River valley, southern Alaska.

Beliaeva (1954) observed larvae in one of 3 lynx examined from the Belovezha Forest.

Lynx feed upon small mammals, mainly hares.

Leporidae.

Larvae were recorded from 2 (3.8 percent) of 53 snowshoe hares, *Lepus americanus dalli* Merriam; these 2 animals contained 0.5 and 3.0 larvae per gram of tissue. Forty hares, including those infected, were collected in the Brooks Range; the remaining 13 were taken in southern Alaska, near Anchorage.

The presence of the larvae in hares, ordinarily herbivorous animals, may possibly be the result of their feeding upon carrion, as they reportedly do when their population is at a high density.

Ochotonidae.

Thirteen pikas, *Ochotona collaris* (Nelson), were collected in the Talkeetna Mountains in southern Alaska; tissues processed were negative.

Sciuridae.

One ground squirrel, *Citellus undulatus* (Pallas), of 129 examined (0.8 percent) was infected. This animal, from St. Lawrence Island, had 0.3 larvae per gram of tissue.

Although primarily herbivorous, *Citellus undulatus* also consumes mammalian flesh when available (Geist, 1933; Cade, 1951). We have observed this behavior in northeastern Alaska and in the Talkeetna Mountains, where remains of voles were found in stomachs and cheek pouches of these squirrels. Such animals feeding upon carcasses of dogs or arctic foxes would of course be exposed to infection.

Four (4.3 percent) of 94 red squirrels, *Tamiasciurus hudsonicus* (Erxleben), were infected. Larvae ranged in number from 0.1 to 1.2 (av. 0.7) per gram of tissue. Three of the infected red squirrels were taken in the Brooks Range, and one in the Copper River valley.

The red squirrel in omnivorous. We have observed red squirrels feeding upon the flesh of the snowshoe hare (carrion), and mammalian bones are often found among food items stored by these animals for winter use. They no doubt eat any warm-blooded animal they are able to capture and kill. Red squirrels scavenge around settled areas, as well.

In addition, the following sciurids were examined with negative results: Marmot, *Marmota m. caligata* (Eschscholtz), 9; Flying squirrel, *Glaucomys sabrinus* (Shaw), 1.

Cricetidae.

Of 18 brown lemmings, *Lemmus sibiricus trimucronatus* Merriam, examined,

one (0.8 percent) from the Brooks Range was infected, with 0.1 larvae per gram of tissue.

The brown lemming is herbivorous, but may eat mammalian tissue under some conditions. This was not seen during the mass die-off occurring along the Arctic Coast in 1949 (Rausch, 1950), but Thompson (1955) observed the consumption of dead lemmings by others during the lemming emigration near Barrow in 1953.

Of 49 red-backed voles, *Clethrionomys rutilus dawsoni* (Merriam), 2 (4 percent) animals from the upper Kenai Peninsula harbored larvae. The numbers of larvae present were 0.5 and 0.7 per gram of tissue.

This vole has a varied diet, and may consume mammalian tissue. In Alaska, it is often found, especially in winter, in and around buildings, where it feeds on stored foodstuffs.

One (1.8 percent) of 57 narrow-skulled voles, *Microtus miurus muriei* Nelson, taken in the Brooks Range, was infected. Little is known of the diet of this vole, beyond the fact that it feeds upon vegetation and stores quantities of leaves and rhizomes for winter use.

Another microtine rodent, the muskrat, *Ondatra zibethica* Linnaeus, was found infected. One (0.9 percent) of 113 animals harbored 1.3 larvae per gram of tissue. This animal was taken in the Copper River valley. The muskrat is seasonally very important as human food in some parts of Alaska.

In addition to the above, the following rodents were examined with negative results: Collared lemming, *Dicrostonyx torquatus rubricatus* (Richardson), 7; Field vole, *Microtus pennsylvanicus* Ord, 10; Tundra vole, *M. oeconomus* Pallas, 234. Roth (1950) examined 4 specimens of *Dicrostonyx* with negative results.

Castoridae.

Twenty-nine beavers, *Castor canadensis* Kuhl, were examined. One of these, taken on Kalgin Island, Cook Inlet, (3.4 percent) harbored 3.0 larvae per gram of tissue.

Erethizontidae.

No larvae were found in 3 porcupines, *Erethizon dorsatum myops* Merriam.

Monodontidae.

Tissues from 49 white whales, *Delphinapterus leucas* (Pallas), were processed, and one animal (2 percent) killed at Wainwright was found infected. Only 17 larvae were obtained in 25 grams of tissue (0.6 per gram).

Thorborg *et al.* (1948) pointed out that the history of an outbreak of trichinosis in 1947 at Kekertak, West Greenland, suggested the source of infection to have been a white whale. Roth (1949) found no larvae in 27 white whales. Brown *et al.* (1949) examined 9 white whales from the Canadian Northwest Territories and found all negative for *Trichinella* larvae. In his summary of the work in Greenland, Roth (1950) reported negative results from a total of 91 white whales.

The white whale feeds largely upon fishes (Vibe, 1950; Vladykov, 1944). It is difficult to understand how this animal might be infected unless it be through the consumption of fishes or invertebrates which have recently ingested mammalian flesh, as postulated by Vibe to explain infection of seals.

Balaenidae.

Samples of striated muscle were obtained from 15 baleen whales killed at Barrow and Wainwright. Although designated as "bowhead" whales by the Eskimo, the identifications were not confirmed. As expected in baleen whales, all were negative. Roth (1950) examined several whales of various species, with negative results.

Odobaenidae.

Fifty-one walrus, *Odobenus rosmarus divergens* (Illiger), from St. Lawrence Island and the Arctic Coast, were examined. No larvae were found.

Thorborg *et al.* (1948) concluded that the flesh of walrus was the probable source of infection in certain outbreaks occurring among Eskimo in West Greenland in 1947. These writers stated (p. 789), "It is generally accepted that this sea mammal lives only on shells, fishes etc. However, it must be mentioned in this connection that remnants of seals have been found in the stomachs of walrus. It is possible that a voracious animal such as the walrus will eat any kind of meat or carrion." Several reports in the literature make it clear that seals are frequently eaten by walrus. A thorough investigation of the feeding habits of the walrus in Greenland has been reported by Vibe (1950).

Roth (1949) reported on the examination of 133 walrus from West Greenland, in which no larvae were found; in a later report, however, (1950) he observed 2 infected animals in 207 (0.9 percent). Brown *et al.* (1949) examined 7 walrus from the Canadian Northwest Territories; all were negative. Kuitunen (1954) reported finding 17 infected walrus among 394 collected in arctic Canada. Fifteen of the infected animals were taken off Southampton Island, and the other 2 at Cape Dorset.

Phocidae.

Samples of tissue from 310 seals, from the Arctic Coast and St. Lawrence Island, of the genus *Phoca* were processed. These are considered as a group, since specific identifications in some cases were not made. The majority was comprised of 2 species, the ringed seal, *P. hispida* Schreber, and the harbor seal, *P. vitulina* Linnaeus. A few ribbon seals, *P. fasciata* Zimmermann, were also included.

Trichinella larvae were recorded from 2 seals (0.6 percent of the total). The animals were from St. Lawrence Island and Point Lay and contained 75.0 and 1.2 larvae per gram of tissue, respectively.

Since *Phoca* spp. feed largely upon invertebrates, the mechanism by which they are infected is not easily understood. Vibe (1950) concluded that seals may consume amphipods feeding around mammalian carcasses which find their way into the sea, and are thus infected either through accidental ingestion of the tissue itself or through mechanical transfer via the amphipods.

One of 52 ringed seals (1.9 percent) was found infected in West Greenland by Roth (1950). Seals of other species, some not occurring in Alaskan waters, gave negative results. No larvae were found in several seals from northern Canada examined by Brown *et al.* (1949).

Of 126 bearded seals, *Erignathus barbatus* Erxleben, one (0.8 percent) was infected. Most of these animals were killed on the Arctic Coast; the infected specimen was taken at Point Lay.

Thorborg *et al.* (1948) first reported trichinosis in a bearded seal. Brown *et al.* (1949) examined a few bearded seals, but none was infected. Roth (1949) found larvae in one of 28 bearded seals from West Greenland; in 1950, he reported no additional larvae after examining 56 animals.

According to Vibe (1950), the bearded seal is relatively omnivorous, feeding upon many species of invertebrates and upon fishes.

Otariidae.

Muscle tissue from 4 sea lions, *Eumetopias jubata* (Schreber), taken on St. Lawrence Island, were examined with negative results. This animal is piscivorous.

Dr. Carlton M. Herman, Patuxent Research Refuge, Laurel, Maryland, examined 101 fur seals, *Callorhinus ursinus cyanocephalus* (Walbaum), from St. Paul Island, Pribilofs, for *Trichinella* larvae, and kindly granted permission for inclusion of his data here. With the exception of one 10-year old bull, all of these animals were young males, 2-4 years old. No larvae were found, using both direct examination and digestion methods.

Fur seals are piscivorous.

Ungulates, Introduced Commensals, and Birds.

In addition to the 2433 mammals discussed above, a few ungulates were also examined: Moose, *Alces alces gigas* Miller, 2; Caribou, *Rangifer tarandus stonei* Allen, 8; Goat, *Oreamnos americanus* (Blainville), 2; Bison, *Bison bison* (Linnaeus), 6. All were negative for *Trichinella* larvae.

The brown rat, *Rattus norvegicus* Berkenhout, is abundant in a few Alaskan towns and generally throughout the Aleutian Islands. It is not known to be feral except in the Aleutian Islands and on some of the smaller islands around Kodiak. A total of 261 rats from Nome, Fairbanks, Kodiak, and Adak Island was processed; of these, 28 (10.7 percent) were infected. Rats of all ages, except young in the nest, were included; a higher prevalence rate would be evident on the basis of adult animals only.

The following flesh-eating birds were also processed: Herring gull, *Larus argentatus* Pontopiddan, 2; Glaucous-winged gull, *L. glaucescens* Naumann, 2; Short-billed gull, *L. canus brachyrhynchos* Richardson, 3; Glaucous gull, *L. hyperboreus* Gunnerus, 6; Pomarine jaeger, *Stercorarius pomarinus* (Temminck), 10; Long-tailed jaeger, *S. longicaudus* (Vieillot), 2; Short-eared owl, *Asio flammeus* (Pontoppidan), 1; Hawk owl, *Surnia ulula caparoch* (Müller), 4; Great horned owl, *Bubo virginianus* (Gmelin), 2; Snowy owl, *Nyctea scandiaca* (Linnaeus), 6; Goshawk, *Accipiter gentilis* (Linnaeus), 3; Sparrow hawk, *Falco sparverius* Linnaeus, 1; Golden eagle, *Aquila chrysaetos* (Linnaeus), 3; Bald eagle, *Haliaetus leucocephalus* Linnaeus, 6; Raven, *Corvus corax principalis* Ridgway, 47; Canada jay, *Perisoreus canadensis* Linnaeus, 3; Magpie, *Pica pica hudsonia* (Sabine), 1. Of these, a few larvae were recorded only from a Pomarine jaeger, collected at Barrow. Until confirmed experimentally, however, this finding is regarded as an error.

TRICHINOSIS IN MAN IN ALASKA

When this work was begun, it was believed by some that undiagnosed disorders reported in arctic villages might be attributable to *Trichinella* infections. There

was no tangible evidence to support this view, however. Lack of medical records in Alaska and limited medical facilities in remote regions made it difficult to obtain information on the prevalence of specific diseases. This situation has been greatly remedied in recent years, and the importance of *T. spiralis* as a parasite of man can best be assessed sometime in the future.

Williams (1946) reported 2 outbreaks of trichinosis. The first of these involved 3 white men, and resulted from their eating the poorly cooked flesh of a brown bear, *U. arctos,* at Yakataga, Southeastern Alaska. In the second, a young Eskimo died at Selawik in February, 1946, after consuming the flesh of either a brown or a polar bear. In view of the time of the year, it is probable that the latter was involved. Further details on this case have not been available.

Three outbreaks of trichinosis are known to have occurred during the period of nearly 7 years covered by the present work.

In the summer of 1950, 2 white women became infected following the consumption of the flesh of a black bear, *U. americanus,* at Nenana, Alaska. Diagnosis was made from clinical evidence and on the basis of the history. The patients were treated by a private physician, and recovery was uneventful.

In the winter of 1954, an Eskimo from the lower Kuskokwim region was treated for trichinosis at the 5005th Air Force Hospital at Anchorage. A high eosinophilia was present, and muscle tissue obtained by biopsy contained numerous, still unencysted larvae. The patient stated that he had eaten only the flesh of moose during the few weeks preceding his illness. This information was kindly provided by Dr. James G. Bridgens, formerly pathologist at the hospital, now at the Independence Sanitarium and Hospital, Independence, Missouri.

In only one instance were several people involved. In May, 1954, a young Eskimo shot a brown bear near Solomon, on the Seward Peninsula. Eight persons in his family, including himself, and a guest consumed the slightly cooked flesh of this animal. Except for the father of the family who died during the interim, all became seriously ill between 2 to 3 weeks following consumption of the flesh. All were treated by Dr. Fred M. Langsam, Maynard-MacDougall Memorial Hospital, Nome, Alaska, to whom we are indebted for this information. Typical symptoms of trichinosis were present in all 7 cases. The hunter himself was the most seriously ill of the group, and was also the last to become ill. An eosinophilia of 26 percent was present, and on June 30 he demonstrated a strong reaction to intradermally-injected *Trichinella* antigen. There were no deaths.

Although the objective of the work reported herein was to determine the prevalence of *T. spiralis* in mammals other than man, intradermal tests were performed on a small number of Eskimo, as follows:

Locality	*Total Number*	*Number Positive*	*Percent Positive*
St. Lawrence Island	232	21	9
Wainwright	71	20	28
Anaktuvuk Pass	17	1	5.8

The people tested at Wainwright ranged in age from 16 to 79 years (average age: 37). Using the same antigen (Trichinella Extract Lederle), Brown *et al.* (1949b) observed positive reactions in 91 (46 percent) of 195 persons tested on Southampton Island. Of 98 persons tested further, 39 (39 percent) gave a positive precipitin test, while 25 of these showed a positive reaction with the intradermal test. At Igloolik, N. W. T., using the same commercially-prepared antigen, Brown

et al. (1950) obtained 22 positive reactions among 100 persons tested. In view of the findings among Canadian Eskimo, the rate of infection at Wainwright, as suggested by the intradermal test, is not remarkably high.

Hitchcock (1950) observed positive intradermal reactions in only 6.6 percent of 150 Eskimo tested in the lower Kuskokwim region. Later work at Kotzebue revealed only 1.6 percent positive reactors among 300 Eskimo (Hitchcock, 1951).

Viable larvae must be frequently ingested by the Eskimo, although present-day methods of food preparation generally preclude extensive outbreaks such as have occurred in Eskimo of Greenland.

DISCUSSION

Conditions in arctic regions are particularly favorable for the transmission of *Trichinella* larvae among wild mammals. Mammals are abundant, although the number of species represented is relatively small. A large proportion of the species present is comprised of those which are entirely or occasionally carnivorous, and predator-prey relationships are in general well defined.

The question of when and how *T. spiralis* became established in mammals in arctic regions has been the subject of some discussion (see Connell, 1949; Roth, 1950). The view that this nematode may have been introduced by man in recent times must be altogether untenable. As pointed out by Cameron (1950), ". . . it is a parasite of carnivores and this development cycle has tended to confine its natural distribution to holarctic boreal regions where carnivores habitually eat carnivores." And further, ". . . animal husbandry has enabled it to become an important parasite of man and pigs in temperate regions." The occurrence of this nematode in man, in some of his domesticated animals, and in his commensals seems now to be entirely independent of the natural cycle occurring in wild mammals, and the latter exists independent of man's influence.

In Alaska, the risk of infection to the aboriginal peoples is correlated with local practices regarding preparation of food. Bears probably constitute the greatest potential source of human infection, but the flesh of these animals is customarily well cooked. Dogs are no longer used as human food in Alaska, nor are foxes or wolves under ordinary conditions. However, such animals as wolverines and minks are still eaten in remote regions, where larger mammals are rare. Large species of marine mammals are potential sources of more extensive outbreaks, since one infected carcass may be divided and consumed by several families. Presently, however, the Eskimo and Indians appear to be no more frequently infected than is the white man who may, through ignorance or carelessness, fail to cook adequately the flesh of bears and other animals.

The mechanism of transmission of *T. spiralis* larvae in wild mammalian populations is not well understood. Eaters of carrion may often become infected, but the place of important prey species—i.e., microtine rodents, sciurids, etc., is not clear. In attempting to learn how the larvae are transmitted to red foxes in Germany, Söntgen (1939) had 40 microscopic examinations performed on each of a total of 521 field voles (*Microtus*) killed in a region where infected foxes were frequently taken. His results were negative. He also reviewed the work of others who had found no larvae in these animals, and concluded that foxes become infected through consumption of the carcasses of other foxes.

Beliaeva (1954) examined more than 700 mouse-like rodents, 126 shrews, and 20 moles from the Belovezha Forest in eastern Poland. Among the small rodents were species of *Clethrionomys, Microtus, Arvicola, Micromys,* and *Apodemus,* as well as house mice (*Mus musculus* Linnaeus).[4] Two shrews (see above) were infected. Larvae were found in 4 of 41 specimens of *Apodemus flavicollis* Melchior, taken around settled areas. An additional 194 of these rodents, taken in forested areas, were negative. Eight of 16 moles, *Talpa europaea* Linnaeus, taken in populated areas, were infected, and the identity of the nematode was confirmed by experimental infection of white mice. Beliaeva found carcasses of carnivores in areas where the infected animals were trapped, and believed that such carcasses, brought by hunters to populated areas and discarded, serve as the source of infection for small mammals as well as for swine.

The occurrence of *T. spiralis* in small rodents and insectivores may depend largely upon the availability of the infected tissue of carnivorous mammals in the form of carrion. In settlements in or near wilderness areas, carcasses left by hunters may have importance, as suggested by Beliaeva. Where the mammalian fauna is abundant, as in Alaska, small mammals may become infected in nature. In Alaska at the present time, it appears that carnivores most frequently become infected through feeding on the carcasses of other carnivores. The investigation of this problem to determine what place the small mammals may have in the transmission of *T. spiralis* in Alaska is being continued.

ACKNOWLEDGMENTS

This opportunity is taken to express appreciation to those who have extended valuable assistance in the course of this work: Personnel of the U. S. Fish and Wildlife Service in Alaska provided numerous specimens and assistance; laboratory facilities and logistic support were provided by the Arctic Research Laboratory, Office of Naval Research, at Point Barrow in the years 1949–53; Dr. Vilhjalmur Stefansson encouraged this work and assisted by making available his correspondence with other investigators in arctic regions on the subject of trichinosis; the late Dr. Hans Roth, Royal Veterinary and Agricultural College, Copenhagen, gave valuable information and suggestions during the early years of this investigation; Mr. Ralph B. Williams, Director of Laboratories, Alaska Department of Health, Juneau, permitted use of data from his records; many of the Eskimo and Indians of arctic Alaska assisted in the collection of mammals, and particular thanks are expressed to the people of Anaktuvuk Pass for their whole-hearted cooperation over a period of several years.

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RELATIONSHIP OF SIZE OF INOCULUM TO TIME TO DEATH IN MICE INFECTED WITH *TOXOPLASMA GONDII*[1]

DON E. EYLES AND NELL COLEMAN

It is a well-recognized fact that the time to death of mice inoculated with *Toxoplasma gondii* bears a generally inverse relationship to the size of the inoculum; however, we have seen no quantitative study relating time to death to the counted number of free organisms inoculated. Likewise, few data are available defining the LD50 inoculum for *T. gondii.* Such information might provide a means for making comparative measurements of the virulence of the various strains of *Toxoplasma* which are being studied in the laboratory. This paper summarizes recent studies by our laboratory on the above subject.

METHODS

The principal strain of *T. gondii* used in the experiments, which has been designated the N.R. strain, was isolated from the Norway rat by this laboratory in 1950 and has been maintained since then by twice-weekly mouse passage. The other strains were sent to us through the courtesy of Dr. Leon Jacobs, National Institutes of Health, Bethesda, Maryland. The mice used were of the N.I.H. general purpose strain and weighed about 20 grams each.

To obtain the desired data, preparations of mouse peritoneal exudate containing numerous *Toxoplasma* were diluted with serum or with physiologic saline in order to obtain parasite suspensions in which the number of organisms varied by a factor of 10. Hemacytometer counts were made so that we could calculate the actual number of free organisms injected. These suspensions were injected intraperitoneally into groups of mice, usually 12 mice at each inoculation level. Fluid was generally taken on the third day of the infection.

Mice so inoculated were observed and the time to death calculated. Mice dying were examined to determine if *T. gondii* were present. Surviving mice were sacrificed three to six weeks after inoculation. Tissues from some were injected into clean mice to see if latent infections were present.

FINDINGS

Relationship of size of inoculum to time to death.

Three experiments were conducted with *Toxoplasma* of the N.R. strain suspended in human serum which had been previously shown to have no anti-toxoplasmic effect when used in the dye test procedure. The organisms were counted by means of a hemacyclometer and varying numbers inoculated intraperitoneally. The mean time to death as related to inoculum size is presented in Table 1 for the experiments alone and combined.

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TABLE 1.—*Relationship of size of inoculum to time to death in mice inoculated with the N.R. strain of* Toxoplasma gondii. *Organisms suspended in human serum. Twelve mice at each inoculation level in each trial*

Number of organisms inoculated	Mean days to death of mice			
	Trial number			All trials (mean)
	1	2	3	
200,000	5.4	4.9	5.5	5.3
20,000	6.6	5.8	5.8	6.1
2,000	6.9	6.8	6.7	6.8
200	7.1	7.1	7.3	7.2
20	8.0	8.3	8.1	8.1
2	Not all mice died of toxoplasmosis			

The larger inocula produced death in a shorter interval in all three experiments. Curves representing the relationship as a linear regression of time to death on the logarithm of the number of organisms inoculated were found to fit well both for the experiments individually and for the pooled data. Analysis of variance show no significant difference between trials so it is believed that the curve based upon the combined data is well representative of the relationship. This curve, $Y = 8.91 - 0.67\ X$, is shown graphically in Figure 1, Y representing the mean time to death and X the logarithm of the number of free organisms inoculated.

Four experiments were also conducted in which the same procedures were followed but the organisms were suspended in physiologic saline solution. In these experiments four different strains were used as designated in table 2 which summarizes the experiments.

TABLE 2.—*Relationship of size of inoculum and time to death in mice inoculated with various strains of* Toxoplasma gondii. *Organisms suspended in physiologic saline. Each group of mice consisted of 12 individuals*

Size of inoculum	Mean days to death of mice			
	N.R. strain	C.J. strain	C.H. strain	Strain 113
2,000,000		4.6	4.7	5.0
200,000	5.3	5.5	6.0	6.3
20,000	6.5	6.6	6.5	6.7
2,000	8.1	7.3	6.9	7.6
200	9.0	8.8	8.0	8.9
20	Not all mice died of toxoplasmosis			

In each of these cases linear regressions of time to death on the logarithm of the number of organisms inoculated were calculated, resulting in the following equations:

$$\text{N.R. strain, } Y = 12.05 - 1.27\ X$$
$$\text{C.J. strain, } Y = 10.95 - 1.02\ X$$
$$\text{C.H. strain, } Y = 10.45 - 0.91\ X$$
$$\text{Strain 113, } Y = 10.81 - 0.91\ X$$

Whether or not the small differences in the above relationships were due to strain or to experimental conditions cannot be determined without additional data; however, it was clear that the variations were not great and that it was possible that only a single population was being sampled.

Figure 1 shows graphically the relationship between the number of organisms injected and the time to death as related to the diluent used. In the case of the organisms suspended in serum it can be seen that death was produced materially

more quickly than by organisms suspended in saline, particularly in the case of the smaller inocula. Likewise it should be pointed out that in no case did mice die of toxoplasmosis when saline suspensions contained two or less organisms; whereas the serum-suspended organisms frequently produced injection at this level or below. These observations indicate a deleterious effect of the saline solution such as has been described by others (Jacobs *et al.*, 1952).

From the figure it can be noted that the two curves diverge from a common

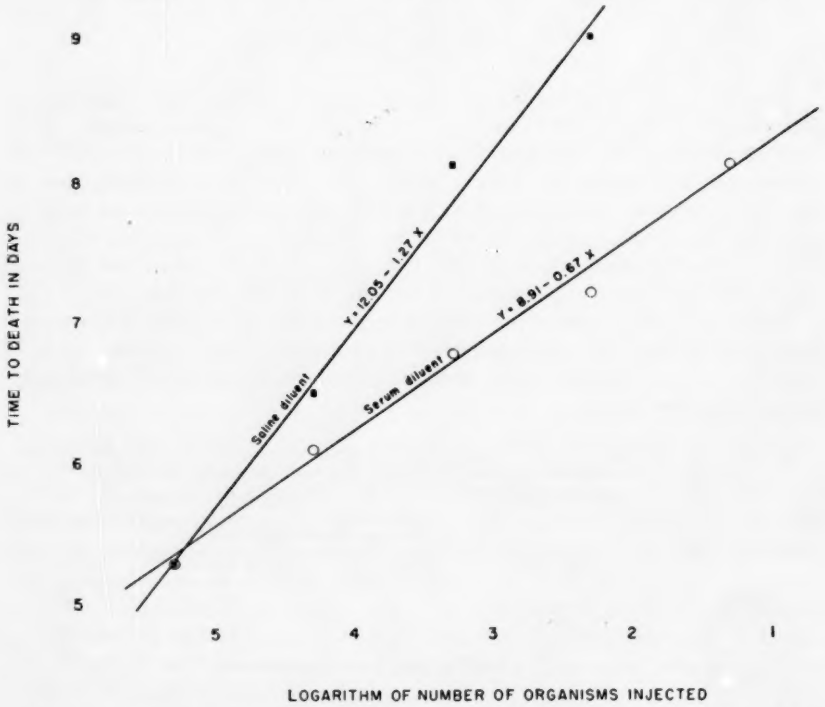

FIG. 1. Linear regression of time to death in days to logarithm of the number of free *Toxoplasma* organisms injected intraperitoneally into laboratory mice. In the case of the serum diluent each point of the curve was determined by a group of 36 mice; in the case of the saline diluent there were 12 mice per group.

origin instead of being parallel as might be expected if the saline killed some definite proportion of the organisms. This may possibly be due to the fact that the less dilute inocula contained appreciable amounts of protein material from the mouse host which may have had a protective effect which disappeared when dilution became great.

Previously it was mentioned that the hemacytometer counts of this study were made of free *Toxoplasma* organisms. The peritoneal fluid contains in addition to numerous free organisms some parasitized exudate cells. The influence of these uncounted parasites in these cells on the relationships we have derived is not known but is believed to be minor due to their small number.

Possibly more significant than the absolute relationship is the relative relationship defined by the slope of the curves. For instance, with organisms suspended in serum a 31 × increase in the number of organisms is necessary to decrease the time to death by one day. If time to death depends upon the organisms reaching some certain density in the tissues of the host, it is possible that this relationship is indicative of the multiplicative capacity of the particular strain of *I. gondii* under study. This relative relationship would not be affected by the uncounted intracellular parasites.

LD50 defined in terms of the number of organisms inoculated.

Data from which the LD50 can be determined were also obtained from the experiments described in the preceding section of this report. Table 3 presents the proportions of mice dying of toxoplasmosis which were given inocula of varying sizes. From these, LD50's were obtained graphically through the use of logarithmic probability paper.

TABLE 3.—*Relationship of the proportion of mice dying to the number of* Toxoplasma *organisms inoculated intraperitoneally into white mice*

	Number of organisms inoculated						
	2×10^5	2×10^4	2×10^3	2×10^2	2×10^1	2×10^0	2×10^{-1}
Proportion of mice dying							
N.R. strain-saline diluent (LD50 = 30)	12/12 (100%)	12/12 (100%)	11/12 (91.7%)	11/12 (91.7%)	5/12 (41.7%)	0/12 (0%)	—
N.R. strain-serum diluent (LD50 = 1)	36/36 (100%)	36/36 (100%)	36/36 (100%)	36/36 (100%)	36/36 (100%)	27/36 (75%)	9/36 (25%)

The LD50 for the organisms suspended in serum was approximately 1 compared with 30 for organisms suspended in saline. This would appear to indicate that a single *Toxoplasma* organism can induce a fatal infection when the organisms are suspended in a favorable medium; however, this conclusion is subject to some qualification. Previously we called attention to the fact that the counts were made of free *Toxoplasma* organisms and that the peritoneal fluid contains some parasitized exudate cells.

Our data indicated not only that the minimum lethal dosage of *Toxoplasma* was very small but that the minimum lethal dosage produced death in mice in 10 to 11 days. This was evidenced by the fact that with two exceptions all mice which died of toxoplasmosis succumbed by the eleventh day. The two exceptions which received a calculated inoculum of 0.2 of an organism died on the 14th and 17th day of the infection.

The experiments also demonstrated that in most cases the minimum lethal dosage was identical to the minimum infective dosage. The basis for this conclusion was that latent *Toxoplasma* infection could be demonstrated in only one of 22 mice which had received small inocula and which survived until sacrificed on the 21st day of the infection. Even in this case, death might have occurred from toxoplasmosis had this mouse been observed longer.

DISCUSSION

Definition of the relationship between the number of organisms injected and time to death provides a measure of virulence of strains of *T. gondii* and as such

might be useful in parasitologic investigations of this protozoan particularly in making comparisons of strains. Likewise, the straight line relationship between the logarithm of the number of organisms injected and the time to death provides the investigator with a yard stick for the estimation of the number of organisms present in experimental inocula containing unknown numbers of parasites of the same strain.

As an example of the latter use of the data, we frequently have occasion to pass blood or other tissues from experimentally infected animals to mice in order to determine parasite density. An estimate of the actual number of organisms in the blood can be obtained by means of the curves of Figure 1. Since the organisms in the blood seldom occur in sufficient numbers to be counted visually, the only alternative to the use of the relationship to time of death is to conduct dilution experiments to determine LD50 levels. This last procedure requires a great deal more work than the method of estimation we are proposing, and gives only a relative measurement.

The LD50 calculations indicate that the white mouse is extremely susceptible to *Toxoplasma* infection. Even though we have not assessed the effect of the few parasitized cells present in the inocula, it is obvious that with the strain we used only a few if not one organism may set up a fatal infection.

SUMMARY

The relationship between time to death and size of inoculum for *Toxoplasma gondii* is well described by a linear regression on the logarithm of the number of free organisms injected intraperitoneally into white mice. Organisms suspended in serum caused death even when a calculated two or less were injected but a saline diluent appeared to have a deleterious effect. In the case of the serum diluent a 31-fold increase in the size of the inoculum was necessary to decrease the time to death by one day. The LD50 inoculum for organisms suspended in serum was in the neighborhood of one organism. The minimum lethal dose was similar in magnitude to the minimum infective dose.

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OBSERVATIONS ON VECTORS OF SCHISTOSOMIASIS MANSONI KEPT OUT OF WATER IN THE LABORATORY. II.*

LOUIS OLIVIER** AND FREDERICO SIMÕES BARBOSA†

Brumpt (1941) and Barbosa and Dobbin (1952) showed that *Australorbis glabratus,* like many other fresh-water pulmonates, is able to live out of water for long periods under certain conditions. In the course of studying the natural history of *A. glabratus* and *Tropicorbis centimetralis,* both of which are vectors of *Schistosoma mansoni* in Northeastern Brazil, Olivier and Barbosa (1955a) (1955b) observed that *T. centimetralis* could also survive out of water, and they described the conditions under which both species lived when their natural habitat became dry.

In conjunction with these field studies on the survival of the schistosome vectors in dry habitats, the snails were also studied in the laboratory. The results of some of these experiments revealed that the vectors can live out of water for long periods when kept in unglazed clay jars at relatively high humidities, and that there appeared to be strain differences with regard to the ability of these vectors to do this (Olivier, 1956).

In another series of experiments an attempt was made to reproduce, in the laboratory, conditions similar to those that occur in temporary bodies of water at the end of the rainy season when the water level falls, the habitats dry, and the snails are left out of water for long periods. This was done by putting the snails in shallow water over a layer of mud and letting the water level fall until the mud was exposed and later dried. The snails were observed under these simulated field conditions. The results of these experiments are reported here.

MATERIALS AND METHODS

The snails used were collected from natural habitats near Recife, Pernambuco, Brazil and maintained during the course of the experiments in large wooden boxes about 150 cm long, 50 cm wide, and 20 cm deep (Figure 1). In two experiments moist soil from the locality in which the snails were collected was placed in the box and the snails scattered over the surface of the soil. The box was kept in the laboratory, and the soil was allowed to dry. In the other experiments, with one exception, soil from the snail habitat was placed in the box, and the latter was then submerged in a tank of water so that the water level was higher than the soil level. After this, the snails were added and the water level was allowed to fall gradually. (In Experiment 6, the water was added immediately after adding the snails.) When the water level had fallen below the soil level, the box was removed from the

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tank and placed in the laboratory where the soil continued to dry. Within one month from the start of the experiments, the soil-water content always fell below 5 percent, and in almost all cases it was below 2 percent. Moreover, the soil-water content was always essentially the same at all levels in the boxes.

At regular intervals a portion of the soil in each box was marked off, removed from the box, and examined for snails. The sample area was removed layer by layer, the snails recovered from each layer were kept separate, and all were tested for viability. The soil usually cracked as it dried, and a few snails from the surface may have fallen into these cracks. Moreover, although the soil samples were removed with great care, it is possible that a few snails at or near the soil surface could have fallen back into the box as one of the superficial layers was removed.

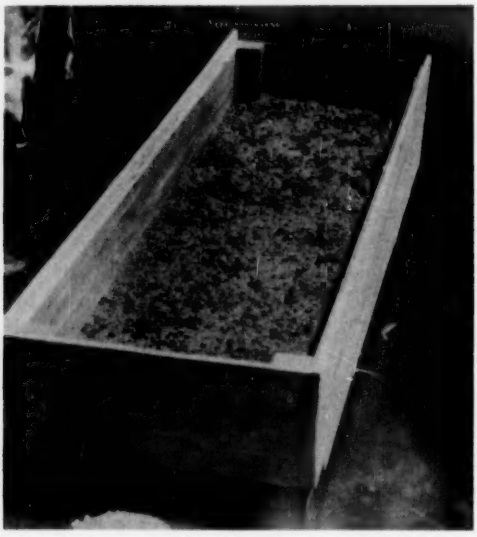

FIG. 1. The box used in Experiment 6. The photograph was taken just before the water level in the tank was raised to cover the soil in the box.

These accidents may have affected the results of the experiments slightly by raising the number of snails recorded from the deeper layers.

The snails were usually tested for viability by passing a beam of light through the shell. With this method, an experienced person could separate the living from the dead snails with a high degree of accuracy. There was some tendency, especially with *Tropicorbis*, to judge dead snails as alive. Therefore, some of the snails were tested by putting them in water for 24 hours, and others were crushed and examined under the dissecting microscope. Samples tested by the last two methods are so indicated in the text.

The boxes of snails were all kept in the laboratory where they were protected from the direct sun and from drastic changes in temperature and humidity. The laboratory temperature ranged from 25 to 28° C in the wet season (March or April to August or September) and from 27 to 30° C during the remainder of the year. The laboratory humidity was more variable, but it usually ranged from 75 to 90

percent during the wet season and from 65 to 75 percent during the dry season.

The snails were always used within two days of the time of collection, and all were taken from localities with low prevalence of trematode infections. Snails known to be infected were never used.

FOUR EXPERIMENTS WITH *Tropicorbis centimetralis*

Experiment 1.

On August 26, 1952, about 4,000 specimens of *T. centimetralis,* 4 to 9 mm in diameter and averaging 5.7 mm in diameter, were collected from the river in Vitória de Santo Antão, a town about 40 kilometers west of Recife. The river flows throughout the year, but its depth and volume fluctuate greatly with the seasons.

A wooden box was filled to a depth of about 6 centimeters with wet mud from the stream bed. Some of the snails were then mixed into the soil while the remainder were scattered over the surface of the mud. Many of the snails put on the soil surface withdrew into their shells, but others moved about actively, and as a result, some moved to the sides of the box and into the corners, while others became partly covered by the mud. The mud dried rapidly and within two days all snails had stopped moving. Within one week the mud was hard and dry and had numerous cracks, and the water content fell to about 2 percent where it remained for the remainder of the experiment.

The first sample from the box was examined about three months after the experiment began. The sample was removed in such a way that the snails recovered could be classified into those in the upper third of the soil, the middle third, and the bottom third. Other samples from the box were examined in the same way 4, 5, 6, 7, 9, and 12 months after the start of the experiment except that the snails were classified into four groups as follows: (1) those that could be removed without disturbing the soil, (2) those in the top, (3) middle, and (4) bottom third of the soil. All the snails were tested for viability by the light method.

Forty-five percent of the snails were on the surface, 44 percent were in the top third of the soil, 5 percent in the middle third, and 6 percent in the bottom third. Since some of the snails had been mixed into the soil, it is not possible to determine how many of the snails might have penetrated the mud, but the number could not have been large.

After seven months, 81 percent of the snails collected were alive. After nine months, 36 percent were alive and after 12 months, 7 percent. The snails collected from the deeper portions of the soil had no better survival record than those collected from the surface.

Experiment 2.

For the second experiment, begun April 8, 1953, the snails were collected from certain small streams near Paudalho. These streams have large seasonal fluctuations though they do not dry up. Mud from one stream bed was put in a box to a depth of about 3 cms, and the box was then immersed in water so that the water level was about 2 cms. above the soil level. About 5,000 snails, 4 to 9 mm in diameter (average 6.4) were placed in the water. The wet mud was very soft and light, and soon after they were put into the water, snails were observed to push into the mud so that they became partly covered. The water level was allowed to fall

gradually over a period of three days. At the end of this period there was no standing water in the box, and it was moved indoors. The soil continued to be very wet for several more days and some of the snails moved about during this time. A large proportion of the snails were still visible on the surface when the soil finally became dry about one week after the box was moved indoors.

Samples were taken from the box, in the manner described for the later samples in exp. 1, at approximately monthly intervals for seven months. Sixty-one percent of all the snails found were collected from the surface. Thirty-five percent were taken from the top third of the soil and most of these were so close to the surface that at least part of the shell was exposed. One percent of all the snails were in the middle soil layer, and 3 percent in the bottom layer. On the basis of the light test, it was found that 45, 109, and 201 days after the start of the experiment 26 percent, 9 percent and 3 percent respectively were alive. The survival pattern of the snails in the deeper layers was the same as that of the snails on the surface.

Experiment 3.

Snails from the river in Vitória de Santo Antão were used. On August 5, 1953, a box was filled to a depth of about 2 centimeters with mud from the river bed. The box was placed in water so that the water level was above the soil. About 5,000 snails, 4 to 10 mm in diameter, were then put into the water. The water level was allowed to fall gradually, and in eight hours there was no standing water in the box, but the mud remained wet and soft for two more days. While the water was present and in the two days that followed its disappearance, the snails moved about actively, and some became partly covered by the mud, apparently as a result of normal activity. One week after the start of the experiment, the soil was firm but somewhat moist to the touch, and all visible snails were inactive and retracted into their shells.

Samples were taken from the box at approximately monthly intervals. First, the snails free on the surface were collected, and then the top and bottom halves of the soil were removed and the snails in them collected. Eighty-five percent of the snails were on the surface, 13 percent were in the top half of the soil and 2 percent in the bottom half. All the snails collected 279, 310, 341, and 380 days after the start of the experiment were tested in water, and the percentages of living snails recovered in the four samples were 8, 20, 7, and 13 respectively.

Experiment 4.

Snails, collected February 17, 1954 from the River Capibaribe near São Lourenço, were used in the final experiment with *T. centimetralis*. The snails were taken from the stream bed below a dam where there is a more or less even flow of water throughout the year.

A box filled to a depth of 3 centimeters with mud from the river was submerged in water so that the water level was 3 centimeters above the soil level. About 3,000 snails, 4 to 9 mm in diameter, were placed in the water, and the water level was then allowed to fall slowly for 24 hours. At the end of this time there was no standing water in the box, but the mud was very soft and wet. Many of the snails were active as the water level fell and some were seen to burrow in the mud apparently as a result of normal feeding activity. However, most of the snails stayed at the surface and did not appear to make any effort to enter the soil when the water dis-

appeared. The soil dried slowly, and 11 days after withdrawal of the water the water content was still 17 percent, but during March, it fell to about 2 percent.

Samples were taken from the box at approximately monthly intervals, and the snails were collected and classified into five categories: (1) those free on the surface, (2) those less than half covered, (3) those in the top, (4) middle, and (5) bottom third of the soil. The distribution of the snails in the 5 categories was: (1) 47 percent, (2) 31 percent, (3) 17 percent, (4) 4 percent, and (5) less than 1 percent (two snails). Again it was noted that most of the snails in the top third of the soil were actually very close to the surface, and that many were partly exposed. The snails collected 15, 43, 83 and 107 days after the start of the experiment were tested for viability in water, and the percentage found alive in these collections were 70, 67, 44, and 18 respectively.

The ratio of living to dead snails was calculated for each category in the four collections mentioned above. Examination of this record showed that there was no evidence to indicate that snails, completely covered by the mud, were more successful in surviving than were the snails free on the surface.

EXPERIMENTS WITH *Australorbis glabratus*

Three experiments, similar to those just described, were done using *Australorbis glabratus.*

Experiment 5.

Snails were collected from the moist pavement of a public water fountain in Olinda on September 25, 1952. A box was filled to a depth of about 3 centimeters with mud collected from a ditch near the fountain. Some of the snails, 2 to 15 mm in diameter, were mixed with the soil, but most of them were placed on the surface. The soil dried slowly and the snails on the surface were active until the soil became too dry. Samples of the box contents were taken 90, 124, 153, and 182 days later, and all the snails recovered were tested by the light method. The percentages of snails found alive in the successive collections were 11, 5, 3, and 0. Of all the snails recovered, 93 percent were free on the surface, 5 percent in the top half of the soil, and 2 percent in the bottom half. Since snails had been mixed into the soil at the start of the experiment, it was not possible to be sure how many had penetrated into the soil, but the number could not have been great.

Experiment 6.

Snails collected from a drainage ditch in Paulista were used.

Heavy, black soil from the ditch, containing much humus, was put in a box to a depth of about 3 centimeters. About 4,000 snails, 14 to 16 mm in diameter, were scattered over the surface of the soil and then the box was immersed in water so that the water level was about 3 cm above the soil level. Most of the snails moved about actively and some burrowed into the very soft mud so that they became at least partly covered. The water level was allowed to fall slowly, and it was six days before the surface of the mud was completely exposed. The experiment was begun on January 17, 1953, and on February 7, the soil was soft, friable, and somewhat moist to the touch. At the end of February, the moisture content of the soil had fallen to about 2 percent, and it remained at this level until the end of the experiment.

Samples were taken from the box at intervals of about one month and the snails recovered were classified into three categories: (1) those free on the surface, and those not more than half covered, (2) those in the top half of the soil including those at the surface but more than half covered, and (3) those in the bottom half.

Of the snails recovered, 37 percent were in the first category, 60 percent in the second category, and 2 percent in the third. Most of the snails in the second category were close to the surface.

The experiment ran for 154 days, and six samples were examined during this time. The snails of the first three samples were examined by the light method while those of the remaining collections were tested by crushing them and examining them under the dissecting microscope. The percentages of snails surviving in the successive collections were as follows: 90, 73, 64, 32, 2, and 6. The snails in the deeper layers of the soil did not live longer than those at the surface, and it was concluded that the location of the snails did not have an important effect on their chances for survival, under the conditions of the experiment.

Experiment 7.

In the last experiment, snails from Bairo Novo, Olinda, were used. The pool from which the snails came never dries, but its level fluctuates with the seasons.

On February 14, 1954, a box was filled to a depth of 3 centimeters with mud from the snail habitat. The box was then flooded and about 2,000 snails, 11 to 22 mm in diameter, were put into the water. The water was 3 to 4 cm deep for six hours, and it was then lowered to 0.5 cm for 13 hours. The water was then drained from the box, and the soil was allowed to dry. While the mud was soft and wet, the snails moved about on the surface and some burrowed into the mud so that they became partly covered. In one week the soil was dry to the touch and friable. After three weeks its moisture content was about 2 percent.

Samples were withdrawn from the box at approximately monthly intervals until 245 days had elapsed. The snails were located in the samples as follows: 62 percent were free on the surface, 37 percent were not more than half covered, 0.5 percent were in the top half of the soil, and none was in the bottom half.

The snails were all tested for viability by the light method, and it was found that many snails died soon after the start of the experiment. After 75 and 100 days 20 percent and 6 percent respectively were alive. In the three samples taken between 145 and 245 days after the start of the experiment less than 1 percent were alive, and all of these were found at or near the surface.

In the foregoing experiments, snails embedded in the dry soil survived about as well as those exposed on the surface. Since snails may become buried under field conditions, and since the mud or soil in the field may be very wet, it was of interest to learn how well the snails could survive burial under such conditions. Therefore, three experiments (nos. 8, 9, and 10) were done in which snails were buried in mud saturated with water.

Experiment 8.

Twenty specimens of *A. glabratus* were put on the bottom of each of three shallow clay jars. Mud saturated with water was then poured over the snails until the depth of the mud in the first jar was 1 cm, the depth in the second jar was 2 cm, and the depth in the third was 3 cm. The next morning snails were found on the

surface of the wet mud in all three jars. Obviously, the snails were able to emerge from the mud, but it may be that they used the walls of the jars as a means for doing this.

Experiment 9.

Twenty specimens of *A. glabratus* were put on the bottom of each of three clay jars. The snails in the first jar were covered to a depth of 3 centimeters with a mixture of wet mud and sand which had the consistency of thick paste. The snails of the second jar were covered to the same depth with the same mixture, but water was then added until the mud was saturated. The snails in the third jar were left uncovered, but the atmosphere about them was kept humid. All three jars were kept on the bench top for 13 days. The soil in the first jar dried somewhat, but it remained moist to the touch until the end of the experiment. The soil in the second jar was kept saturated with water, and the third jar remained at a relative humidity of about 90 to 95 percent. At the end of the 13 days, all the snails in the first jar were found on the bottom under the soil, and all were alive. All the snails in the second jar were dead, and none had moved from its original position. Fourteen of the 20 snails in the third jar were alive.

Experiment 10.

The failure of the snails to survive under water-saturated mud provoked a further test. Twenty specimens of *A. glabratus* were put on the bottom of a metal can 17 cm in diameter and 25 cm deep. Heavy black mud saturated with water and of such a consistency that it could be poured was added to the can until it was 15 cm deep. Two days later one snail was found on the surface of the mud next to the side of the can. It was felt that this snail had escaped from the mud by crawling up the side of the can. Four days after the start of the experiment, the contents of the can were examined, and one living snail was found in the top third, three dead snails were found in the middle third and the remainder of the snails were found dead on the bottom of the can.

DISCUSSION

The experiments just described provide additional information concerning the survival time of the two schistosome vectors when they are kept out of water. Small numbers of *A. glabratus* lived five months, but it is probable that if snails from the temporary pools of Paulista had been used (Olivier and Barbosa, 1955a), longer survivals would have been recorded. *T. centimetralis* tended to live longer than the *A. glabratus* under essentially the same conditions. Moreover, the *T. centimetralis,* in these experiments, lived longer than they did when collected from the water and kept in clay jars. Experiment 3 in Olivier (1956) is more or less comparable with Experiments 1 and 3 in the present paper, and Experiment 5 in Olivier (1956) is directly comparable with Experiment 4 of the present paper. In each case the snails kept in the wooden boxes survived longer than those in the clay jars. On the other hand, *A. glabratus* kept on the soil in wooden boxes lived about as long as those kept in clay jars (Olivier, 1956). Some of the experiments are not directly comparable since the snails used in most of the experiments were from widely different habitats and may have represented strains with differing capabilities for living out of water, but Experiment 14 of Olivier (1956) and

Experiment 7 of this series are directly comparable, and the survival patterns in the two experiments were not significantly different.

In the experiments described here, as the water level fell, the snails continued their apparently random movements over the soft mud and many were observed to become partly covered with the mud as a consequence of their activity. The snails did not respond to the disappearance of the water by entering the mud. On the contrary, almost all the snails in every experiment were left stranded at, or just below, the surface.

A small proportion of the snails were recovered from the deeper soil layers, and this is clear evidence that some snails penetrated one, two, or even three centimeters into the mud before it dried. The factors causing these snails to enter the mud are unknown, but since only a small number of the snails did this, it is believed that this behavior is atypical and not necessarily related to the fall in the water level. Moreover, it must be remembered that the mud was very soft and loose in these experiments and that, consequently, conditions for penetration of the mud must have been particularly favorable. The mud of natural habitats is usually more firm and dense and so probably offers less favorable conditions for penetration.

The observations related to soil penetration are in agreement with others made in the field (Olivier and Barbosa, 1955a, b) where it was observed that aestivating *A. glabratus* and *T. centimetralis* were almost always found at or near the soil surface in the protection of vegetation and debris and not deep in the soil. It may be noted in this connection that Barlow (1933, 1935) found that *Biomphalaria boissyi* and *Bulinus truncatus* made little effort to escape drying by entering the mud. Also, Blacklock and Thompson (1924) found that *Physopsis* sp. from Sierra Leone tended to remain on, or just under, the surface of soft mud as the water was withdrawn.

On the other hand, a number of workers have considered it possible, or probable, that vectors of *S. mansoni* and *S. haematobium* may penetrate the mud to avoid drying or the effects of poisonous chemicals (Cawston, 1927; Archibald, 1933; Berry *et al.*, 1950; Watson, 1951; Barbosa and Dobbin, 1952; and others). Moreover, Veloso (1953) has stated that in Minas Gerais, Brazil, *A. glabratus* frequently lives in the mud and that some specimens can be found deeply embedded. This subject has had little critical investigation and much further work must be done in diverse endemic areas before this point is clarified.

Although pulmonate schistosome vectors may penetrate the soil in very small numbers only, it is certain that large numbers of these snails may be accidentally buried. Snails may be buried in the stream bed under deposits of silt or they may be buried under soil or debris by falling banks and stream clearance operations. Others may be pressed into the mud under the feet of men and animals.

The experiments reported here show that if snails are embedded in relatively dry soil they may live there for a long time. Similarly, Cawston (1929) has found that some African schistosome vectors could live up to 30 days when buried in garden soil.

The snails lying in the soil survived about as long as those at the soil surface, indicating that their location in the soil did not affect their chances for survival. This may have resulted from the fact that the soil water content was about the same at all levels in the boxes. Under field conditions, the deeper soil layers are usually

more humid than the surface soil. Therefore, since high humidity apparently protects snails against excessive water loss (Olivier and Barbosa, 1954) snails buried in some natural habitats might survive longer than those at the surface of the soil.

However, burial does not always favor the snails. The three experiments in which snails were buried under wet mud showed that although the vectors in Northeastern Brazil may be able to move to a limited extent in very soft, water-saturated mud, they cannot survive long in this environment. Perlowagora-Szumlewicz and Oliveira Dias (1955) have reported results of more extensive experiments of this type. They found that *A. glabratus* (*Australorbis immunis*) buried in wet mud to a depth of 2 to 8 centimeters for 24 hours, always died. von Brand *et al.* (1950) have shown that *A. glabratus* will live only about 16 hours without oxygen. Since wet mud may be nearly anaerobic, this may explain the rapid death of the snails in this environment.

It should also be borne in mind that although some buried snails may survive for long periods, they may not be able to escape by their own efforts. This being the case, snails in the mud or soil must die eventually unless uncovered in some way.

SUMMARY

Australorbis glabratus and *Tropicorbis centimetralis*, the snail hosts of *Schistosoma mansoni* in Northeastern Brazil, are able to survive long periods out of water under certain conditions. The snails were put on wet mud or in shallow water over mud and allowed to dry gradually. Snails so dried lived for many weeks and some lived over one year. The snails did not tend to enter the mud to avoid the consequences of drying, and most were found on the surface. A small percentage entered the mud, but this appeared to be atypical and was not necessarily a response to unfavorable conditions. Snails embedded in dry soil lived about as long as those on the surface. Snails embedded in mud saturated with water died in a short time.

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RESEARCH NOTE

HABITAT OF *GIARDIA* IN THE KITTEN

While examining the tissue sections of a kitten for morphological stages of *Isospora felis*, the presence of a natural infection of *Giardia* was noted.

Since *Giardia* trophozoites of other animals are usually recorded as being in the small intestine, it was of interest to determine the location of this organism in kittens.

This *Giardia*-infected kitten, wt. 900 gm., was sacrificed at the age of 64 days. Representative tissues were removed from the junction of the small intestine and the stomach, at the first two inches of the small intestine and every three inches of the remainder of this organ. The cecum was cut into four pieces. Similar portions were removed from every inch and one-half of the large intestine. The portions of the intestine for the microscopic examinations were fixed in Zenker's solution, infiltrated with paraffin, sectioned at a thickness of 7 microns and stained by the routine hematoxylin eosin staining technique.

The entire stained cross-sections were systematically examined using the oil immersion objective. *Giardia* trophozoites were found throughout the small intestine, cecum, and large intestine, except at the junction of the small intestine and the stomach. The trophozoites seemed to be more numerous in lower portion of the small intestine with as many as eleven stages being found per oil immersion field. At four different locations trophozoites were found outside the lumen of the gut in the mucosa. They did not appear to be intracellular, but superimposed on the epithelial cells.

No *Giardia* cysts were found on examining the stained tissues, wet saline mounts of intestinal scrapings, or excreta from this *Giardia infected kitten.*—DOROTHY J. HITCHCOCK, *Clemson Livestock Laboratory, P. O. Box 1174, Columbia, S.C.* and THOMAS D. MALEWITZ, *Department of Anatomy, Michigan State University, E. Lansing, Michigan.*

NEW HOSTS FOR TREMATODES OF THE GENUS *HETEROPHYES* IN EGYPT[1]

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I. NEW DEFINITIVE HOSTS

Trematodes of the genus *Heterophyes* Cobbold, 1866, have been known to be endemic in the Nile Delta for many years. More than 100 years have passed since *Heterophyes heterophyes* (v. Siebold, 1852) was first reported as an intestinal parasite of Egyptians, yet less is known of the epidemiology of *Heterophyes* than of any other human helminth parasite in Egypt.

Early descriptions of species of this genus were provided by Looss (1894, 1896, 1902), who described 8 species and 2 subspecies from Egyptian hosts. Later, Witenberg (1929), after revising the family HETEROPHYIDAE, discarded a number of species of doubtful validity from the genus *Heterophyes* and recognized only 3 species as being valid, namely: *H. heterophyes* (v. Siebold, 1852), *H. dispar* Looss, 1902, and *H. aequalis* Looss, 1902.

Khalil (1937), in his description of the life history of *Heterophyes heterophyes,* named a brackish-water snail, *Pirenella conica,* as the first intermediate host, several species of fishes, *Mugil* spp. and *Tilapia* spp., as the second intermediate host and man, dog and cat as the final host.

In 1953, a survey of trematodes of the genus *Heterophyes* of Egyptian birds and mammals was begun in this laboratory. The present paper gives the results of that survey and reports new definitive hosts for each of the 3 species of *Heterophyes* studied.

MATERIALS AND METHODS

Collection and examination of vertebrates was carried out from 6 October 1953 to 15 January 1955. The majority of wild animals were collected either from the vicinity of Lake Burullus, a large body of brackish-water in Fouadiya Province near the Mediterranean, or at a brackish-water lake (Lake Qarun) in the Faiyum Depression some 50 miles southwest of Cairo.

Domestic dogs and cats were obtained from widely separated localities including the environs of Lakes Manzala, Burullus, Idku and Qarun, as well as from the city of Cairo. Collection sites are shown in Figure 1.

Twenty-three species of wild and domestic mammals and 15 species of birds were examined during the survey. Only those species which normally feed on fish or those suspected of occasionally eating fish were included. While it is doubtful that bats of the several species listed feed on fish, they are included as a matter of interest since Macy (1953) reported *Heterophyes heterophyes* from a bat, *Rhinolophus clivosus acrotis,* collected in the Yemen.

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The worms recovered from the small intestine of the various hosts were fixed in FAA and stained with Semichon's acetocarmine. All measurements were made from stained whole mounts and are given in millimeters. Identifications are based on keys and descriptions of the various *Heterophyes* species as given by Witenberg (1929).

RESULTS

Heterophyes spp. were found in the following species of vertebrates: *Milvus migrans aegyptius* (Egyptian kite), in 4 of 17 specimens; domestic cat, in 73 of 79 specimens; *Felis chaus nilotica* (wild cat), in 1 of 7 specimens; domestic dog, in 15 of 23 specimens; *Canis aureus lupaster* (jackal), in 3 of 5 specimens; *Vulpes vulpes aegyptiaca* (Egyptian fox), in 11 of 55 specimens, *Rattus rattus* (house rat), in 1 of 44 specimens.

Species of vertebrates not infected with *Heterophyes* spp., with the number of each species examined, are as follows: *Corvus corone sardonius* (hooded crow), 39; *Pandion haliaetus haliaetus* (osprey), 1; *Nyroca fuligula* (tufted duck), 9; *Podiceps nigricollis nigricollis* (blacked-necked grebe), 11; *Podiceps cristatus cristatus* (great crested grebe), 2; *Calidris alpina schinzii* (dunlin), 2; *Calidris minuta* (little stint), 1; *Tringa totanus totanus* (common redshank), 2; *Tringa nebularis* (greenshank), 3; *Tringa ochropus* (green sandpiper), 3; *Tringa glareola* (wood sandpiper), 2; *Capella gallinago gallinago* (common snipe), 4; *Larus ridibundus ridibundus* (black-necked gull), 6; *Gallinula chloropus chloropus* (moorhen), 2; *Fulica atra atra* (coot), 7. *Crocidura olivieri* (shrew), 26; *Rousettus aegyptiacus* (fruit bat), 4; *Nycteris thebaica thebaica* (bat), 15; *Otonycteris hemprichi hemprichi* (bat), 1; *Pipistrellus kuhli kuhli* (bat), 11; *Taphozous perforatus perforatus* (bat), 62; *Taphozous nudiventris* (bat), 11; *Rhinopoma microphyllum* (bat), 2; *Rhinopoma hardwichei cystops* (bat), 63; *Tadarida aegyptiaca aegyptiaca* (bat), 5; *Tadarida teniotis ruppelli* (bat), 23; *Herpestes ichneumon ichneumon* (mongoose), 8; *Fennecus zerda* (fennec fox), 5; *Mustela nivalis subpalmata* (weasel), 10; *Mus musculus* (house mouse), 47; *Acomys cahirinus* (spiny-back mouse), 10.

The 3 species of *Heterophyes* recorded during the survey are considered separately.

Heterophyes heterophyes (v. Siebold, 1852)

Synonyms: *Distoma heterophyes* v. Siebold, 1852; *D. heterophyes hominis* Diesing, 1855; *Dicrocoelium heterophyes* Weinland, 1858; *Fasciola heterophyes* Moquin-Tandom, 1860; *Heterophyes aegyptiaca* Cobbold, 1866; *Mesogonimus heterophyes* Railliet, 1890; *Coenogonimus heterophyes* Looss, 1899; *Cotylogonimus heterophyes* Lühe, 1899; *Heterophyes nocens* Onji and Nishio, 1915; *H. pallidus* Looss, 1902; *H. fraternus* Looss, 1902; *H. heterophyes sentus* Looss, 1902; *H. persicus* Braun, 1901.

New Hosts: *Felis chaus nilotica* de Winton; *Vulpes vulpes aegyptiaca* Sonnini; *Canis aureus lupaster* Hemprich and Ehrenberg.

Previously Reported Hosts: Man; Domestic Cat; Domestic Dog; Rabbit (experimentally); 'Persian Wolf'; *Circaetus gallidus; Pelecanus onocrotalus; Milvus migrans aegyptius; Rhinolophus clivosus acrotis.*

This was the most common of the 3 species of *Heterophyes* recovered from the small intestine of the carnivores examined. Domestic dogs and cats were heavily infected, as many as 760 worms being recovered from the small intestine of a single dog. Foxes, collected in the vicinity of Cairo (Giza Province) and at Baltim on Lake Burullus, were infected. Worms of this species were also recovered from the small intestine of two jackals and one wild cat from the Lake Qarun area.

Of the several species of birds examined, only the kite (*Milvus* sp.) was infected with this species.

The measurements obtained during this study agree with those of previous authors. Our findings support the conclusion of Witenberg (1929), that there is considerable variation in size of adult worms of this species, depending on the species of host. The average measurements obtained from a minimum of 10 worms selected at random from each host species are given in Table I.

Heterophyes aequalis Looss, 1902

Synonyms: *Distomum fraternum* Looss, 1894; *Heterophyes inops* Looss, 1902.
New Hosts: *Rattus rattus* (L.); *Felis chaus nilotica* de Winton; *Canis aureus lupaster* Hemprich & Ehrenberg.
Previously Reported Hosts: Domestic Cat; Domestic Dog; 'Persian Wolf'; *Pelecanus onocrotalus; Milvus migrans aegyptius.*

Worms of this species were recovered from the small intestine of 1 of 44 house rats examined, also from the domestic dog, domestic cat, jackal, wild cat and kite.

This is the smallest of the known species of the genus, with worms from all hosts averaging less than one millimeter in length. The measurements obtained during the present study do not differ appreciably from those given by Witenberg (1929).

Heterophyes dispar Looss, 1902

New Hosts: *Felis chaus nilotica* de Winton; *Canis aureus lupaster* Hemprich & Ehrenberg; *Vulpes vulpes aegyptiaca* Sonnini; *Milvus migrans aegyptius* (Gmelin).
Previously Reported Hosts: Domestic Dog; Domestic Cat; 'Persian Wolf.'

This species was more common than *H. aequalis,* and in the Lake Qarun area, a greater percentage of hosts was infected with this species than with *H. heterophyes.* During the survey, worms of this species were recovered from the domestic dog, domestic cat, wild cat, jackal, fox and kite. Our measurements of worms of this species agree with those of previous authors.

II. NEW FISH INTERMEDIATE HOSTS

A species of mullet, (*Mugil* sp.), was the first fish suspected of being a source of infection in Egypt with *Heterophyes heterophyes.* Kobayashi (1923) described metacercariae belonging to a trematode of the genus *Heterophyes,* from the muscle tissue of formalin-preserved mullet obtained from the Port Said area.

Later, Khalil (1924, 1937) reported the following species of fishes as second intermediate hosts for *Heterphyes heterophyes* in Egypt: *Mugil cephalus* (Linnaeus), *Mugil capito* Cuvier, *Mugil auratus* Risso, *Tilapia nilotica* Boulenger, and *Tilapia zilli* Boulenger. These fishes were also suspected of being the intermediate hosts in Egypt for *Heterophyes dispar* and *Heterophyes aequalis,* as Witenberg (1929) found the fish hosts to be the same for the 3 species of *Heterophyes* in Palestine.

Early in 1954, a study was begun to determine if additional fishes from the brackish-water lakes of Egypt, serve as a source of infection with *Heterophyes heterophyes.* In addition to this primary objective, it was hoped that the second intermediate hosts for *H. aequalis* and *H. dispar* could be established. The present

paper gives the results of the study, and reports two additional species of fish serving as the intermediate hosts for the 3 species of *Heterophyes* known to occur in Egypt.

MATERIALS AND METHODS

Collection and examination of 7 species of fishes, commonly used as food by the local inhabitants, was carried out from January to November, 1954. These fish were obtained from various brackish-water lakes, including the Mediterranean coastal lakes of Manzala, Burullus, Idku and Maryut; as well as from Lake Qarun in the Faiyum Depression. These lakes are shown in Figure 1.

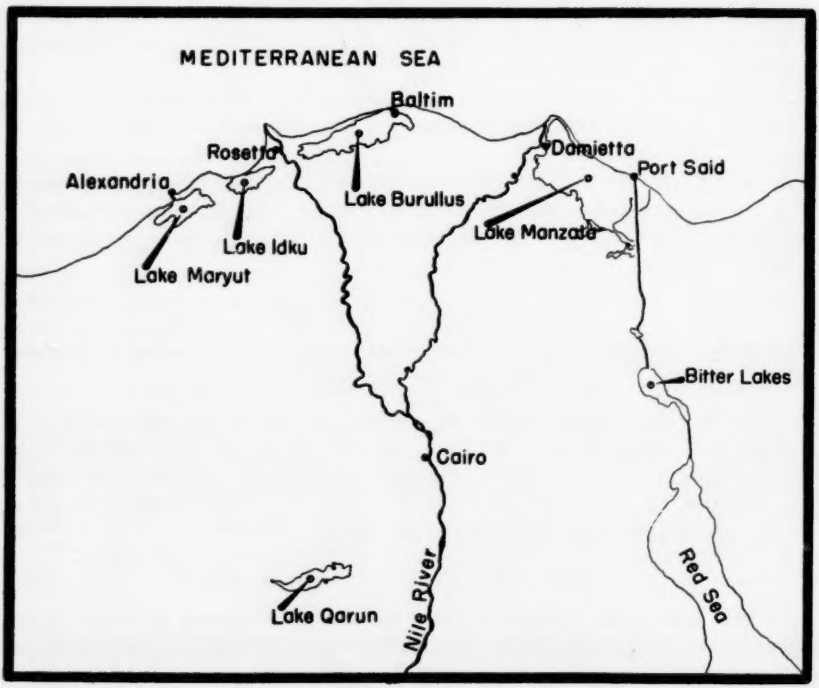

FIG. 1. Map of northeastern Egypt showing collection sites of vertebrate hosts of *Heterophyes* species.

The fish were transported on ice to Cairo where they were examined the following day. The muscle tissue of each individual fish was examined microscopically for the presence of encysted metacercariae after pressing the tissue between 2 glass microslides. The species of fishes collected, with the numbers examined and found infected, are given in Table II.

As it was impossible to identify the species of *Heterophyes* present on the basis of the metacercariae alone, adult worms were obtained for identification by feeding the metacercariae to pups. Six-weeks old pups, born and reared in the laboratory, were each fed 5 grams of infected tissue. These pups were autopsied three weeks after the feeding and the mature worms recovered from the small intestine.

TABLE I—*Average measurements in millimeters of adults of* Heterophyes heterophyes *from Egyptian hosts*

Host	Body Length	Body Width	Width Oral Sucker	Length Oral Sucker	Diameter Ventral Sucker	Width Genital Sucker	Width Pharynx	Length Pharynx	Diameter Testes	Diameter Ovary
Domestic Cat	1.36	.36	.077	.061	.152	.144	.052	.051	.124	.092
Domestic Dog	1.83	.58	.108	.094	.271	.220	.059	.065	.191	.119
Vulpes v. aegyptiaca	1.68	.58	.115	.096	.273	.222	.056	.070	.183	.125
Canis aureus lupaster	1.66	.50	.100	.082	.250	.219	.053	.060	.185	.125
Felis chaus nilotica	1.60	.45	.091	.083	.191	.183	.052	.054	.171	.125
Milvus migrans aegyptius	1.06	.38	.063	.052	.119	.120	.037	.037	.143	.091

TABLE II—*Species of fishes examined for metacercariae of* Heterophyes

Scientific name	Common name (English)	Common name (Arabic)	Locality (Lake)	Number examined	Number infected
Mugil cephalus	Grey Mullet	Bouri	Idku Burullus	114	114
Mugil capito	Thin-lipped Grey Mullet	Tobar	Burullus Manzala	67	61
Mugil auratus	Golden Grey Mullet	Garan	Qarun Burullus	105	105
Tilapia nilotica	?	Bolti Nili	Qarun	108	75
Tilapia zilli	?	Bolti Akhdar	Burullus Manzala	58	39
*Sciaena aquilla**	Shadow Fish	Loot	Burullus Idku	44	20
*Solea vulgaris**	Sole	Samak Mousa	Idku Qarun	84	46

*New host records.

TABLE III—*Results of feeding* Heterophyes *metacercariae to pups*

Fish host	Number of pups infected	Average no. worms recovered	Number of pups infected with		
			(*H. heterophyes*)	(*H. aequalis*)	(*H. dispar*)
Mugil auratus	21	90	19	16	21
Tilapia nilotica	12	6	6	12	10
Solea vulgaris	13	2	2	7	12
Sciaena aquilla	11	24	11	3	1

The adult worms were fixed with FAA and stained with Semichon's acetocarmine. Identifications of the worms recovered, are based on the keys and descriptions of species of the genus *Heterophyes* as given by Witenberg (1929).

RESULTS

The metacercariae of *Heterophyes* spp. were found encysted in the muscle tissue of each of the 7 species of food fishes examined (Table II). Metacercariae from 4 of these fish hosts were fed to pups with the result (Table III) that egg-producing adults of 3 species of *Heterophyes: H. heterophyes, H. dispar* and *H. aequalis,* were later recovered from the small intestine of the experimental host. The results agree with the findings of Witenberg (1929): the fish hosts are the same for these 3 species of *Heterophyes.*

Two of these fishes, *Sciaena aquilla* Cuvier and *Solea vulgaris* (Linnaeus), represent new host records for *Heterophyes heterophyes, H. dispar* and *H. aequalis.*

The 3 species of mullet were more heavily infected, both with regards to numbers of metacercariae per individual fish and percentage of individuals of each species infected. An incidence of 100 per cent infection was recorded for *Mugil cephalus* and *Mugil auratus.* Likewise, as shown in Table III, a greater number of adult trematodes were recovered from pups fed mullet, *Mugil auratus,* than from the pups fed other species of fishes. These findings support the conclusions of Witenberg (1929) and Khalil (1937), that fishes of the genus *Mugil* are the preferred second intermediate hosts for members of the genus *Heterophyes.*

ACKNOWLEDGMENT

The authors are indebted to Dr. Herbert Friedmann, U. S. National Museum, to Abd El Rahman El Bolock, Institute of Hydrobiology, Alexandria, Egypt, and to Harry Hoogstraal, Naval Medical Research Unit No. 3 for providing host identifications. We also wish to thank Said K. Mohamed of the Department of Parasitology, NAMRU-3, for assistance in the collection and examination of host specimens.

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AN ATYPICAL *ORNITHODOROS HERMSI* FROM UTAH (IXODOIDEA, ARGASIDAE)

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Over a period of approximately three years, 73 ticks of the genus *Ornithodoros* have been received from Utah. The ticks were received through Glen M. Kohls of this Laboratory, who has identified them provisionally as *Ornithodoros hermsi* Wheeler, Herms, and Meyer (Beck, 1955).

O. hermsi is a vector of relapsing fever spirochetes in California, Colorado, Idaho, Nevada, Oregon, and Washington and it is the only species of the genus reported from British Columbia where relapsing fever is present. Relapsing fever has also been reported from Utah, but the vector has not been determined, although *O. parkeri,* from which spirochetes have been recovered repeatedly, is present in many areas in this State (Davis, 1941). The ticks from Utah were, therefore, tested for relapsing fever spirochetes by feeding on white mice. Spirochetes were not recovered.

STUDIES IN BIOLOGY AND TRANSMISSION

Rearing

In attempts to rear stock for comparative studies with *O. hermsi* from other areas, much difficulty was encountered in obtaining engorged larvae. Larvae of *O. hermsi* usually attach readily and engorge in a very short time, but in several attempts the larvae from Utah failed to attach to adult white mice, rabbits, guinea pigs, or man. Finally, one lot, placed under a feeding capsule on the clipped belly of a guinea pig, attached, but instead of engorging in the usual few minutes, several days were required as tabulated below.

Number of larvae	*Days required for engorgement*
6	9
25	10
33	11
24	12
10	13
9	14
7	15
114	

It was thought that in the second generation, after the ticks had accepted the mouse as a host through the several nymphal stages, the larvae might also accept the mouse as a host. Therefore, the larvae in the second generation of reared ticks were placed on the clipped belly of an adult white mouse. They failed to attach and again failed to attach to man.

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Transmission of relapsing fever spirochetes

In former studies, *O. hermsi* from the several areas has transmitted *Borrelia hermsii* (Table I). Therefore, these ticks were tested similarly. Ten of the Utah *O. hermsi* and 10 Oregon *O. hermsi,* all in the first nymphal stage, were fed on a mouse infected with *B. hermsii* from California. At the first and second test feedings of the Oregon ticks, there was transmission by seven and 10, respectively. However, there was no transmission by the Utah ticks at either of the two test feedings.

Survival of the spirochete within the ticks

Some species of relapsing fever spirochetes survive for long periods within ticks which do not transmit them. Therefore, 77 days following the infective feeding and nine days following the second test feeding, two ticks were dissected and the tissues were examined by dark-field illumination. Spirochetes were not found

TABLE 1.—*The Transmisson of* Borrelia hermsii *by* Ornithodoros hermsi *from different western states*

B. hermsii	*O. hermsi*				
Strain	**California**	**Colorado**	**Idaho**	**Oregon**	**Utah**
California	(71) 80	(49) 35	(27) 44	(10) 100	(10) 00
Colorado	(40) 60	(21) 100	(9) 88		
Idaho	(56) 72	(40) 55	(22) 77		

Numbers in parentheses are number of ticks tested individually.
Numbers outside parentheses denote per cent transmission.

in any of the tissues examined: Salivary glands, coxal organs, central ganglion, and the ovaries. On the same day, three of the ticks were triturated in saline and the triturates were injected, separately, into white mice, none of which became infected.

DISCUSSION

According to Kohls, (see Beck, 1955) the ticks from Utah are morphologically similar to *Ornithodoros hermsi* from other areas. Biologically, they are also similar in that the nymphs and adults feed rapidly on white mice and at the end of feeding, the coxal fluid is first seen as a white crystalline mass at the external opening of the coxal organs. Of about 40 species of the genus studied biologically, *O. hermsi* is the only one to exhibit this phenomenon. However, the Utah ticks differ from *O. hermsi* from other areas in that: (1) the larvae required 9 to 15 days for engorgement instead of the usual few minutes; (2) they failed to transmit *Borrelia hermsii* from California which was readily transmitted by *O. hermsi* from Oregon, as a control, and from other areas; (3) they failed to retain the spirochete for any considerable period as shown by dark-field examination of the tissues and by injection of the triturates into white mice.

The failure of *B. herimsii* from California to survive within the ticks from Utah is an additional expression of marked variation. Two exceptionally long-time survivals of a spirochete foreign to the tick are *Borrelia hispanica* for five years and *B. duttonii* for six and a half years in *O. nicollei* (Davis and Mavros, 1955).

Ecologically, the Utah ticks also differ from *O. hermsi* from other areas. The collections of these ticks from Utah, reported by Beck (1955), were made from

the nests of *Neotoma lepida* and *N. cinerea*. Three nymphs were also collected from the body of a *N. lepida*. This is the first report of *Neotoma spp.* as hosts to *O. hermsi*. With the exception of the larvae of some species, ticks of this genus engorge rapidly in all stages and the hosts are, therefore, determined for the most part by associations. In the extensive studies in California, spirochetes were recovered from chipmunks and tamarack squirrels in areas where *O. hermsi* was present (Wynns and Beck, 1935). In our initial studies in Colorado (Davis, 1939), *Eutamias spp.* were the only rodents seen in the area. In a second collection (1939), pine squirrel fleas, *Orchopeds caedens* (Jordan), were found in nesting material. In a third collection (1940), a squirrel captured in a nest was identified as *Tamiasciurus fremonti fremonti*. These collections were from Douglas fir "snags." A collection from Placer County, California, in 1941 was made from a hollow log which had toppled from a Douglas fir "snag." In one record from Oregon (Davis, 1941), ticks were collected from a hollow pine log from which a cottontail rabbit escaped as we were approaching the area. Many hollow junipers, which were obviously the home of pack rats, were examined but ticks were not found. There is the interesting observation of Gregson (1948) in British Columbia of *O. hermsi* in the nest of a bluebird, presumably *Sialia currucoides*.

Marked biologic variations within species of the genus have been reported from other areas, more especially in the small form of *O. erraticus* from Egypt (Davis and Hoogstraal, 1954), and in *O. tholozani* (Baltazard *et al.*, 1954) but this is the first record of an extreme variation in *O. hermsi* if the Utah ticks are this species rather than a new species.

SUMMARY

Ticks from Utah, provisionally identified as *Ornithodoros hermsi*, differ biologically from *O. hermsi* from other areas in that the larvae required several days for engorgement, instead of a few minutes, and in that they failed to transmit a California strain of *Borrelia hermsii* which was transmitted readily by *O. hermsi* controls from Oregon, and in other studies from California, Colorado and Idaho. This spirochete failed to survive within the ticks for any considerable period as was shown by dark-field examination of dissected tick tissues, and by the injection of triturates of the ticks into white mice. If the Utah ticks are *O. hermsi* and not a new species, this is the first marked biologic variant in ticks of this genus reported from the Western Hemisphere.

ACKNOWLEDGMENTS

The dark-field examinations were made by Dr. Willy Burgdorfer, of this Laboratory. The pine squirrel fleas were identified by Dr. William L. Jellison, also of this Laboratory. The squirrel from Boulder County, Colorado, was identified by Prof. E. Raymond Hall, then of the Museum of Vertebrate Zoology, University of California at Berkeley.

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RESEARCH NOTE

A PARASITIC COPEPOD (*SALMINCOLA FALCULATA*) ATTACHED TO A FISH HEART

Copepods of the genus *Salmincola* are frequntly found on salmonid fishes attached to the skin, fins, gills, and the membranes of the mouth and branchial cavity. An unusual case of attachment to the heart was found recently during a routine examination of a number of young sockeye salmon, *Oncorhynchus nerka.* The specimen was small compared with individuals found on the skin and gills of other fish from the same sample, but on the basis of the structure of its appendages appears to be *Salmincola falculata* (Wilson).

As shown in the accompanying photograph, the copepod lay dorsal to the heart with one edge of its bulla firmly attached to the wall of the auricle. The location of the body of the parasite is uncertain as the copepod was not noted until the heart had been removed and examined under a dissecting microscope. It may have been present in either the pharynx or

branchial chamber with the pedicel of the bulla passing through the thin tissues which separate these regions from the pericardial cavity. However, the less probable occurrence of the entire copepod within the pericardial cavity cannot be ruled out.

The host was a downstream migrant from Cultus Lake, British Columbia, collected on April 20th, 1955. Its fork length of 8.4 cm. was well within the range of length for other fish in the sample. No evidence of pathological effects was noted.—JAMES R. ADAMS, *Department of Zoology, University of British Columbia, Vancouver, and Pacific Biological Station, Nanaimo, B. C., Canada.*

THE BITING MIDGES OF THE GENUS *CULICOIDES* FOUND IN THE BERMUDA ISLANDS (DIPTERA, HELEIDAE) I. A DESCRIPTION OF *C. BERMUDENSIS* N. SP. WITH A KEY TO THE LOCAL FAUNA

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The biting midges of the genus *Culicoides* are commonly called "sandflies," "punkies," or "no-see-ums." In various parts of the world their great numbers reduce property values, and resort areas frequently must spend considerable time, money and effort to protect their clientele from their vicious bites which frequently are more painful than those of many mosquitoes. In addition, in some areas these midges transmit filariid parasites and virus disease of man and animals.

During the months of June and July, 1955, an initial study was made of the *Culicoides* of the Bermuda Islands to determine what species were to be found there, their breeding habitats, and their potential hazard. In the course of this investigation three species were recovered which included the females of an undescribed species. No males were taken and as a result only the female is described here.

The terminology of wing variation follows that of Tillyard as outlined by Friend (1942) thus, Cu_1 and Cu_2 of some workers became M_{3+4} and Cu_1 respectively, and cell Cu_1 becomes M_4.

In the study of the palpal and antennal segments it was found that a wide individual variation existed in the proportional lengths of the respective segments. As a result, rather than give the proportional lengths of the antennal segments, the antennal ratio (length of last 5 segments divided by length of segments 3 through 10) was determined for 10 specimens selected at random. The extremes and average are given. Of these 10 specimens 2 had the fourth and fifth antennal segments of the left antenna fused. The combined length of these 2 segments was more than one-fourth shorter than 2 normal segments. In a random sample of the palps of 10 specimens, it was found that in 3 the second segment was longer than the third, in one instance as much as one-fifth, while in 2 specimens the third was longer than the second, one nearly by one-seventh. The remaining 2 specimens had segments 2 and 3 of equal length. The proportional lengths given for the palpal segments are, therefore, average lengths, to the nearest whole number, determined from the actual length of the 5 segments of the palps of 10 specimens.

Some difficulty was encountered on my part in determining sensoria on the antennal segments. Wirth reported, in a personal communication, that he found them on segments 3, 13, 14 and sometimes on segment 12. I was able to find them on segments 3, 13 and 14 on 3 of 25 specimens while on the remaining 22 specimens I could find them only on segment 3, if indeed at all.

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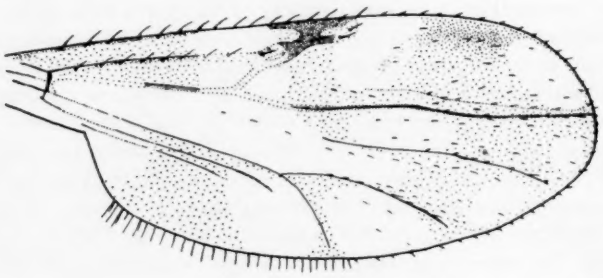
Contribution No. 224 of the Bermuda Biological Station. This study was supported by a National Science Foundation grant-in-aid and a Childs Frick Fellowship from the Bermuda Biological Station.

Culicoides bermudensis n. sp.

Female: Small, brownish, poorly marked. Length 1.15 to 1.32 mm., wing 0.81 to 1.02 by 0.38 to 0.44 mm.

Head dark brown, eyes bare, fairly widely separated, ratio of separation of eyes to diameter of facet in center of eye 12 : 10. Antennae with scape brown, flagellar segments lighter, antennal ratio from 1.04 to 1.18, average 1.10, sensoria on segments 3, 12, 13, 14 on some specimens. Palps (Fig. 2) short, segments in proportion of 14 : 29 : 28 : 17 : 16, third segment with 2 sensory pits (Fig. 3). Mandibles with 4 to 8 teeth, usually 7 or 8.

Mesonotum without markings except for prescutellar spots (similar to *C. canithorax*). Scutellum light brown, postscutellum darker, the border dark brown when wet, pleura light brown. Legs unbanded, light brown throughout except for darker tibial combs. The first of the 4 long spines of the hind tibial comb the longest.



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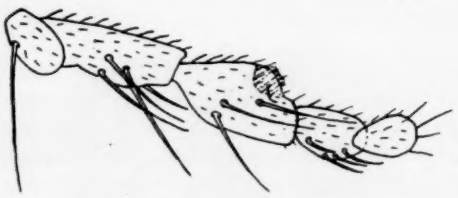

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EXPLANATION OF FIGURES

C. bermudensis

FIG. 1. Wing of female.

FIG. 2. Female palp.

FIG. 3. Third palpal segment as seen in different aspects showing the two sensory pits.

Wing with anterior radial cells complete, costa to 0.55 of wing length; macrotrichia sparse, in distal two-thirds of wing, on wing veins, in rows parallel to wing veins and scattered in cells R_5, M_1, M_2 and a few in cell M_4. Anterior margin of the wing with a light spot over r-m cross-vein extending into long light area in cell M_2, about two-thirds of first radial cell in this light area; cell R_5 with a large light spot at end of costa which cuts across the distal end of the second radial cell, a darker finger-like projection entering this light spot from the distal margin; a second light spot at the distal extremity of cell R_5 the long axis of which lies at a 45° angle with vein M_1; cell M_1 with a proximal elongate light spot and a smaller often obscure light spot at the distal extremity; basal four-fifths of cell M_2 with a long light streak which unites at base with spot at base of anal cell, an oval light spot at distal end of cell, often with a very indistinct border; cell M_4 with a large light spot which opens on the wing margin; anal cell with a large pale spot at distal end of anal veins extending to wing margin and another light spot at cell base which merges with the light area in cell M_2. Halters light brown to creamy in color.

Abdomen dull brownish black, spermatheca one, large, oval—0.134 mm. by 0.088 mm.

Holotype ♀, Trott's Pond, Hamilton Parish, Bermuda Islands, June 13, 1955, R. W. Williams (recovery cage) (type U. S. N. M.). Paratypes 15 ♀♀ same data as type; 15 ♀♀ Pampas Farm (on South shore Marsh) Smiths Parish, June 21–27, 1955.

C. bermudensis belongs to the *crepuscularis* group in the subgenus *Monoculicoides* of Khalaf (1954) which includes *C. alaskensis* Wirth, *C. canithorax* Hoffman, *C. crepuscularis* Malloch, *C. sphagnumensis* Williams and *C. wisconsinensis* Jones from North America. *C. bermudensis* resembles *C. canithorax* more closely than it does any of the others in that the thorax of both lack distinctive markings, and the location of the wing markings are quite similar. However, there are numerous differences between these two species which are as follows:

Morphological Differences	*C. canithorax*	*C. bermudensis*
Antennal ratio	1.2	Less than 1.2.
Number of mandibular teeth	15	4 to 8, usually 7 or 8.
Width of eye separation	2.8 units of measurement.	7.5 units of measurement.
Length of palps	12.5 to 14 units.	7.8 ± units.
Wing length	15.5 units.	9.8 units.
Macrotrichia	Abundant.	Sparce.
Light spots on wings	More sharply defined.	Margins less distinct.
	Spot at end of costa without a darker finger-like projection in it.	Spot at end of costa with darker finger-like projection entering from distal border.
	Long axis of distal spot in cell R_5 lies at less than a 30° angle to vein M_1.	Long axis of distal spot in cell R_5 lies at a 45° angle to vein M_1.
	Spot in cell M_4 occupies less than ½ area of cell.	Spot in cell M_4 occupies more than ½ area of cell.
	Spot in anal cell, at end of anal veins small, not reaching wing margin.	Spot in anal cell at end of anal veins larger, reaching wing margin.
Tibial combs	4 or 5 long spines, second longest.	4 long spines, first longest.
Spermatheca	2.25 units in length.	3.8 units in length.

Characterization of the Genus Culicoides with a Key to the Local Fauna

The species of the genus *Culicoides* are characterized by possessing 2 anterior radial cells in the wing which are approximately equal in length; costa ending at about one-half to two-thirds wing length. Microtrichia dense, macrotrichia always present although they may be nearly lacking in some species; wing either with or without light spots on a darker background. Mesonotum with or without a pattern but always with a pair of sensory pits or depressions near the anterior margin. Antennae of female with first segment obscured, the second larger than the remaining segments, segments 3 to 10 rounded or oval, segments 11 to 15 elongated. First tarsal segment at least twice as long as the second, claws of both sexes simple, small and equal, empodium extremely small.

1. Uniformly light (cream colored) species with unspotted wings ..... *C. floridensis* Beck
   Darker species with spotted wings 2
2. A distinct pattern on the thorax; margin of white spots on wings rather sharply defined; no portion of second radial cell within a white spot; macrotrichia abundant, numerous in anal cell ..... *C. crepuscularis* Malloch
   Thorax unmarked; margin of white spots on wing not sharply defined; a portion of the second radial cell in a white spot; macrotrichia sparse, few in anal cell
   C. bermudensis Williams

ACKNOWLEDGMENT

I wish to thank Dr. W. W. Wirth of the U. S. National Museum for ascertaining that *C. bermudensis* was an undescribed species.

ADDENDUM

Mrs. Elizabeth C. Beck of the Bureau of Entomology, Florida State Board of Health, has a manuscript in press in the *Florida Entomologist* describing the males and females of this species as it appears in Florida. The females from Bermuda and from Florida are quite different in a number of respects, although similar enough that Dr. Wirth believes them to be the same species. These differences between the two geographic forms are discussed in Mrs. Beck's paper.

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THE BITING MIDGES OF THE GENUS *CULICOIDES* IN THE BERMUDA ISLANDS (DIPTERA, HELEIDAE)

II. A STUDY OF THEIR BREEDING HABITATS AND GEOGRAPHICAL DISTRIBUTION*

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Johnson (1913) mentions only two species of the family Heleidae (Ceratopogonidae, formerly in the family Chironomidae) from the Bermudas. One he described as new, *Ceratopogon fur* Johnson, and the other he designated only as "*C.* sp." Ogilvie (1928) does not mention this family as occurring there, nor does Waterston (1940). To date, the biting midges of the genus *Culicoides,* vectors of filariid parasites and virus diseases of man and animals, have never been reported from these islands.

During June and July, 1955, light trap and recovery cage studies were conducted in each of the several parishes (Fig. 1) and observations on breeding habitats were made.

METHODS

Four recovery cages, modified from those described by Dove *et al.* (1932) in that a solid wooden top replaced their muslin top covered by hardware cloth, and a mosquito light trap were utilized. Two recovery cages were placed in each of 15 areas (for the most part the edge of ponds and the margins of marsh drainage ditches) for a period of one week each. When possible an attempt was made to place the two cages in a given area in different types of habitats, usually having a different flora. The light trap was operated for a week in each of 7 different areas and for 4 days at the Biological Station. With the exception of the Biological Station study area the light trap was usually placed a few hundred feet or

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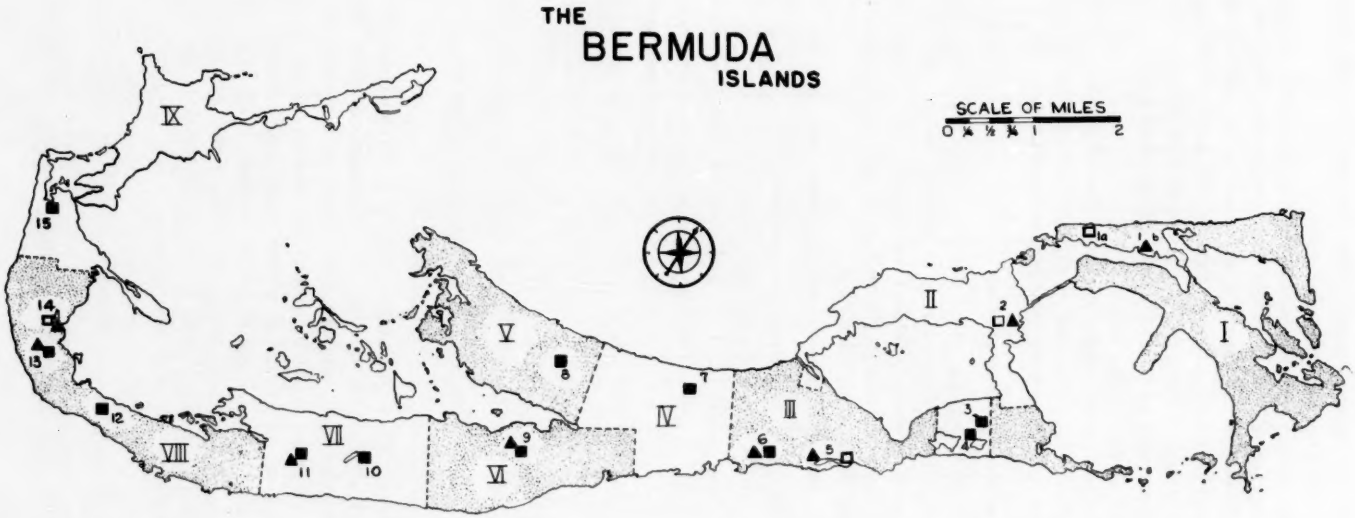

PARISHES

I. St. George's
II. Hamilton
III. Smith's
IV. Devonshire
V. Pembroke
VI. Paget
VII. Warwick
VIII. Southampton
IX. Sandy's

TRAPS

▲ Light Trap, Positive

■ Recovery Cage, Positive

□ Recovery Cage, Negative

LOCATIONS

1a. Lover's Lake
1b. Biological Station
2. Wilkinson Pond
3. Mid-Ocean Gulf Pond (Larger of the two)
4. Trott's Pond
5. Spittal Pond
6. Pampas Farms
7. Devonshire Marsh
8. Pembroke Marsh or Marsh Folly
9. Paget Marsh
10. Warwick Pond
11. Warwick Marsh
12. Seymor Pond
13. Southampton Marsh
14. Evans Pond
15. Pilchard Bay

FIG. 1. Geographical location of *Culicoides* recovery cage and light trap studies on the Bermuda Islands—June and July, 1955.

less from an area being sampled by recovery cages. The jars from recovery cages and from the light trap were examined daily for *Culicoides*.

A Beckman pH meter was utilized for determining the pH of the soil samples from each area studied (an average of 3 readings for each area is the figure used in Table 2) and a set of hydrometers calibrated for determining the salt content of water at 15.4° C. was used to ascertain the salinity of the water from each habitat. Water samples were cooled to about 12° C. in a large cold storage room, brought outdoors and as they warmed up to 15.4° the readings were taken. Soil temperatures were ascertained at each area studied.

The use of a solid top on the recovery cages increased the temperature of the soil covered by a cage as much as 8° C. over that of exposed soil next to the cage with the result that emergence may have been considerably stimulated.

RESULTS AND DISCUSSION

In Fig. 1 are designated the locations where recovery cage and light trap studies were conducted. Indication is also made as to whether or not species of *Culicoides* were trapped at each location. Table I summarizes the information from light trap studies while Table II presents the ecological information obtained on the breeding habitats found positive for *Culicoides*.

TABLE I.—*Number of* Culicoides *caught in light traps—Bermuda Island, June, July, 1955*

Location	Date 1955	C. crepuscularis		C. bermudensis		C. floridensis		Comments
		Males	Females	Males	Females	Males	Females	
Biological Station	6/4 –6 7/26–27	1	0	0	0	0	0	No likely breeding areas in the vicinity.
Wilkinson Pond	6/7 –14	1	0	0	0	3	3	Not found breeding at edge of Pond.
Spittal Pond	6/15–20	1	5	0	0	0	0	Not found breeding at edge of Pond.
Pampas Farms	6/21–27	12	53	0	211	0	0	Both spp. breeding within 100 ft. of Light-Trap.
Paget Marsh	6/28–7/4	67	80	0	2	0	0	Both spp. breeding within 300 ft. of Light-Trap.
Warwick Marsh	7/5 –7/11	6	2	0	0	0	0	Light-Trap about 600 ft. from closest known breeding area.
Southampton Marsh	7/19–25	6	28	0	11	0	0	Light-Trap about 400 ft. from closest known breeding area.
Evans Pond	7/12–18	0	8	0	0	0	0	Not found breeding at edge of Pond.
Total		94	176	0	224	3	3	

Three species were recovered, *C. crepuscularis* Malloch, *C. bermudensis* Williams and *C. floridensis* Beck. The total number of individuals recovered both in the light trap and from recovery cages was very low. Many of the nights were exceedingly windy, a factor which no doubt influenced the number caught in the light trap, and which throughout the course of a year could well influence the total population. Macky (1947) points out that "the frequency of days with strong winds, that is of 30 m.p.h. and over, varies from the winter maximum in February where more than half of the days have winds of this speed, to the summer minimum in July when 4% of the days have a strong wind. Similarly, the frequency of days with gales (mean wind speed over 10 consecutive minutes reaches a value of 39 m.p.h. or more) has a maximum of 25% in February and a minimum of 0.2% in July."

C. floridensis was recovered only in the light trap at one locality near Wilkin-

TABLE II—*Ecological Observations on* Culicoides *Breeding Habitats-Bermuda Islands, June-July, 1955*

Location	Date 1955	pH	Salinity (Pts. per 1,000)	Temperature H_2O	Temperature Soil	Vegetation	No. of Culicoides recovered-Larvae and pupae reared C. crepuscularis Females	C. crepuscularis Males	C. debilipalpis Females	C. debilipalpis Males	Remarks
Trott's Pond	6/13	6.60	31.5	26.3°	24.3°	*Rhizophora mangle*-Mangrove	0	0	49	0	Cages placed on fallen decaying mangrove leaves. Pond level fluctuates with tides and periodically covers breeding areas.
Pampas Farm (South Shore Marsh)	6/21	7.68	15.0		25.5°	*Eleocharis interstincta* - Knotted Spike-Rush *Mariscus jamaicensis* - Prickley Sedge	0	0	0	0	Cages moved twice. Soil saturated with water-none standing.
	6/22	7.00	15.0		25°	*Scirpus validus* - Matrush *Lippia nodiflora* - Cape weed *Sporobolus virginicus* - Seashore Rush Grass	92	57	32	0	No standing water - soil saturated.
Devonshire Marsh	6/21	6.97	1.2		24°	*Commelina longicaulis* - Chicken Grass *Lippia nodiflora* - Cape weed	1	1	0	0	Cages at margin of drainage ditch - not in standing water.
	6/22	7.01	1.2		24°	*Eichornia crassipes* - Water Hyacinth *Paspalum distichum* - Joint Grass	25	20	4	0	Cages at margin of drainage ditch - not in standing water.
Paget Marsh	6/28	6.40	3.9		28°	*Eleocharis capitata* - Capitate Spike Rush *Polypogon littoralis* - Perenial Beard Grass *Sesuvium portulacastrum* - Seaside Purslane	28	37	4	0	Cages in margin of drainage ditch - not in standing water.
Pembroke Marsh or Marsh Folly	6/29	6.50	1.5	26°	25.5°	*Typha* sp. Cat Tail	0	0	0	0	Cage entirely in water.
	6/30	6.60	1.5	26°	26°	*Sporobolus virginicus* - Seashore Rush Grass	2	0	0	0	Cage entirely in water. Soil mostly grass roots.
Warwick Pond	7/5	6.47	25.0	34°	31°	*Sporobolus virginicus* *Eleocharis praticola* - Meadow Spike Rush	0	0	19	0	Cages in standing water.
Warwick Marsh	7/6	6.88	2.4	37°	31°	None	0	0	0	0	Sandy soil at edge of drainage ditch, partially in water.
	7/7	6.48	2.4	35°	31°	*Typha* sp. - Cat Tail *Lippia nodiflorus* - Cape weed	46	26	0	0	Muck soil at edge of drainage ditch, partially in water.
Seymor Pond	7/12	7.01			24°	*Tamarix gallica* - Tamarisk	3	1	0	0	Area damp - never covered by water. Shaded by Tamarisk.
	7/13	7.50	13.0	27°	27°	*Sporobolus virginicus*	1	0	0	0	Cage entirely in water.
Southampton Marsh	7/19	6.34	4.8	30°	28°	*Sporobolus virginicus*	6	10	0	0	Cage in marsh itself.
	7/20	6.32	4.8	32°	28°	*Typha* sp.	452	423	3	0	Cage at edge of drainage ditch Temp. of soil under cage 36°C.
Pilchard Bay	7/19	6.46	36.2	28.2°	28°	*Nerium oleander* - Oleander	1	1	0	0	Cage in mud under Oleanders. Ocean tides covered area.
	7/21	6.73	36.2	28.4°	28°	*Sesuvium portulacastrum* - Seaside Purslane	0	1	0	0	Tides covered area.
Mid-Ocean Golf-Club Pond (Larger of the two)	7/28	6.70	10.7	28°	26°	*Typha* sp. *Sporobolus virginicus*	F*	0	0	0	Cage over small pools of water at pond edge.
TOTAL							658	577	111	0	

son Pond (Table I). Foote and Pratt (1954) state that it is probable that this species is not a salt water breeder. The fact that it was never recovered from habitats which were brackish in nature here seems to confirm this hypothesis.

C. crepuscularis is one of the most common of the inland species in the United States east of the Continental Divide. Edmunds and Keener (1954) found it to be a species which causes a great deal of annoyance to man in certain localities of western Nebraska. Apparently the population in the Bermuda Islands at the

present time is below the critical level necessary to be considered a pest of the human population. Over most of its range in the United States this species is associated with fresh water while in the Bermudas it was found associated with water ranging in salinity from 1.2 to 36.2 parts per thousand.[1] At Pilchard Bay, where the higher concentration of salt in the water was found, the breeding area was covered with sea water by tidal action twice each day. The sex ratio of males of this species to females caught in the light trap was 1 : 1.86 and trapped in recovery cages 1 : 1.14.

Like *C. crepuscularis, C. bermudensis* has a considerable saline tolerance, being found within a salinity range of from 1.2 to 31.5 parts per thousand. Of 224 specimens taken in the light trap and 111 in recovery cages all were females. Sixty-eight of the 111 captured in recovery cages were collected from two areas in which no *C. crepuscularis* were taken. Therefore, at least from these locations there was no possibility of confusing males of *C. bermudensis* with *C. crepuscularis.* All *C. crepuscularis* males from other areas were rechecked and no *C. bermudensis* were found among them.

From Table I it can be seen that 65 specimens of *C. crepuscularis* were taken in the light trap at Pampas Farms while 211 *C. bermudensis* were captured. One might suppose that *C. bermudensis* was breeding in the vicinity in greater numbers. However, as indicated in Table II, 149 *C. crepuscularis* were captured in the recovery cages whereas only 32 *C. bermudensis* were taken in these cages. It seems probably, therefore, that *C. bermudensis* is either more attracted to the light trap than is *C. crepuscularis* or else quite by chance the recovery cages were placed in spots which were not typical of the *Culicoides* population throughout the breeding area.

Williams (1955) found that in Michigan the tolerance of the genus *Culicoides* for extremes in pH was considerable (8.00 – 3.85 = 4.15) but the range of a given species was much less, usually being less than 2 (with one exception, which was 2.21). Here in the Bermuda Islands the pH range for the 2 species trapped was again less than 2, 1.18 for *C. crepuscularis* and 0.69 for *C. bermudensis* (Table II).

SUMMARY

Previous studies by others of the insects of the Bermuda Islands had not disclosed members of the haematophagous fly genus *Culicoides* to be among the local fauna. During the months of June and July, 1955, an introductory study of this genus was made utilizing recovery cages and a light trap in each of the several parishes. Three species were recovered, *C. crepuscularis* Malloch, *C. bermudensis* Williams, and *C. floridensis* Beck. Ecological studies were made on the breeding habitats of the first two named species and differences in geographical distribution noted (Table II). Although *C. crepuscularis* is a pest of man in some areas of the United States, the population of this species on the Bermuda Islands during the summer of 1955 was not sufficiently large to be of any great nuisance value. The host preferences for the other 2 species, which had even lower levels of population, are not known.

[1] Although sea water is usually stated to contain 35 parts per thousand of salt, Mark (1913) found that 6 of 7 readings of surface sea water at Bermuda contained over 35, the high being 36.83.

ACKNOWLEDGMENT

I wish to express my sincere thanks to Dr. William Sutcliffe, Jr., Director of the Biological Station for his kind assistance on a number of occasions, to members of the Bermuda Agriculture Experiment Station, Gordon R. Groves, Director, Idwal Wyn Hughs, Plant Pathologist, J. Hubert Jones, Assistant Horticulturist, and C. A. Baker, Horticulturist, for their helpful assistance in transporting equipment and identification of the plants, to Dr. W. W. Wirth of the U. S. National Museum for the identification of the *Culicoides,* and to Mrs. C. J. Economou, School of Public Health and Administrative Medicine, Columbia University for the production of Fig. 1.

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TWO NEW SPECIES OF TICKS FROM SOEMBAWA ISLAND, INDONESIA (ACARINA: IXODIDAE)

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In 1954 a collection of 1094 ticks taken off 97 *Varanus salvator* (Laurenti) and off 4 snakes (determination unavailable) on Soembawa Island, Indonesia, from April to August, 1948, were sent to the author for study by Professor F. C. Kraneveld, Rijksuniversiteit, Utrecht, Netherlands. These ticks represented two new species, *Aponomma kraneveldi* and *Aponomma soembawensis*. Other species previously recorded from this island were: *Haemaphysalis bispinosa* Neumann, 1897, *H. cornigera* Neumann, 1897, *H. papuana* Thorell, 1882, by Krijgsman and Ponto (1931, 1932), *Rhipicephalus haemaphysaloides pilans* Schulze, 1935, by Krijgsman and Ponto (1931, 1932) and Anastos (1950) and *Rhipicephalus sanguineus* (Latreille, 1806) by Anastos (1950).

Aponomma kraneveldi n. sp.
Male (Fig. 1, A–F)

Body: About as long as wide but narrowed in region slightly below cervical grooves; widest point just anterior to spiracular plate. Fifteen specimens, exclusive of capitulum, from 2.20 to 2.50 mm. long, average 2.36 mm. and from 2.35 to 2.70 mm. wide, average 2.56 mm. Scutum reddish-brown, without ornamentation. Eleven festoons distinct and longer than wide. Lateral grooves visible only as faint depressions under oblique light. Cervical grooves short, deep and convex externally. Punctations few, inconspicuous and limited to scapular regions, lateral margins and posterior region. Dorsal foveae and hairs absent. Ventral body surface lighter in color; with numerous punctations, each containing a short, white hair. Genital opening opposite level of coxae II. Anal opening opposite level of mid-line of spiracular plates. Spiracular plates about two and a half times as long as wide, with prolongation visible dorsally.

Capitulum: Fifteen specimens, from tip of palpi to tip of cornuae, from 0.676 to 0.754 mm., average 0.711 mm.; length of basis from 0.260 to 0.299 mm., average 0. 267 mm.; width of basis from 0.598 to 0.676 mm., average 0.632 mm. Basis sub-rectangular with lateral margins slightly convex. Cornuae small but distinct with posterior border of basis between them straight. Palpi short and rugose with article two slightly less than one and a half times as long as article three. Hypostome length 0.450 mm.; corona large, with slight apical notch; dentition 3/3, with 6 to 8 teeth per file and with very tiny denticles extending three-fourths its length.

Legs: Moderate in length. Coxa I with two short equal spurs; coxae II–IV with single small spur; spur on coxa IV slightly longer than those of coxae II and III.

Female (Fig. 1, G–K)

Body: Three unengorged females, exclusive of the capitulum, 2.25, 2.40 and 2.50 mm. long and 2.25, 2.55 and 2.65 mm. wide, respectively. Body sub-circular and widest at level of spiracles. Dorsal foveae, marginal grooves and hairs absent. Scutum of fifteen specimens from 1.15 to 1.35 mm. long, average 1.26 mm. and from 1.80 to 2.00 mm. wide, average 1.94 mm.; reddish-brown and inornate. Punctations very small and more or less uniformly distributed. Cervical grooves short, deep and convex externally. Festoons in unengorged females similar in appearance and in number to those of male but less distinct in partially engorged specimens and indistinct in fully engorged specimens. Ventral body surface lighter in color; punctations numerous, with each containing a short, white hair. Genital aperture and anal opening in same position as those of male. Spiracular plate similar in general form to that of male but not as long.

Capitulum: Fifteen specimens, from tip of palpi to tip of cornuae, from 0.728 to 0.819 mm., average 0.776 mm.; length of basis from 0.273 to 0.299 mm., average 0.285 mm.; width of basis from 0.624 to 0.715 mm., average 0.669. Basis sub-rectangular with lateral margins more convex externally than those of male; dorsal surface rugose anteriorly and laterally to porose areas.

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Cornuae small but distinct with posterior border of basis between them straight. Distance between porose areas equal to their diameter. Palpi short and rugose with length of article two slightly less than one and one half times length of article three. Hypostome similar to that of male.

Legs: Legs, coxae, coxal spurs and tarsi similar to those of male.

Holotype: Female, from snake, (determination unavailable) Soembawa District, Soembawa Island, July 29, 1948. Deposited in the U. S. National Museum, Washington, D. C., no. 2220.

Allotype: Male, same data as holotype.

In some respects the male of *A. kraneveldi* resembled that of *A. barbouri* Anastos, 1950, a species previously described from Java but it could be readily separated from this species which was also inornate. *A. barbouri* showed numerous punctations which were uniformly distributed and the external spur of coxa I was about twice as long as the internal spur. In *A. kraneveldi* the punctations were absent or so small as to be scarcely visible and the spurs on coxa I were short and equal in length.

Aponomma soembawensis n. sp.

Male (Fig. 2, A–F)

Body: Slightly wider than long and somewhat quadriform; widest at level of spiracles and narrowest at region opposite cervical grooves. Fifteen specimens, exclusive of the capitulum, from 1.80 to 2.10 mm. long, average 1.90 mm. and from 2.05 to 2.40 mm. wide, average 2.20 mm. Scutum dark brown to black and ornate. Ornamentation usually consisting of seven irridescent spots variable in size and in shape. Typically one small spot external to each cervical groove, one in median central region, two small spots at posterior end of scutum and one large lateral spot at each side of scutum. Cervical grooves small, deep and comma-shaped. Punctations large, deep and uniformly distributed but some specimens with fewer punctations in central region of scutum. Lateral grooves, dorsal foveae and hairs absent. Ventral body surface lighter in color; punctations numerous, each containing a short, thick, white hair. Genital opening opposite level of coxae II and III. Anal opening opposite level of spiracular plates. Spiracular plates slightly less than twice as long as wide, with prolongation visible dorsally.

Capitulum: Fifteen specimens, from tip of palpi to tip of cornuae, from 0.676 to 0.819 mm., average 0.749 mm.; length of basis from 0.234 to 0.286 mm., average 0.260 mm.; width of basis from 0.468 to 0.520 mm.; average 0.503 mm. Basis rectangular with lateral margins nearly straight. Cornuae small with posterior border of basis between them slightly sinuous. Palpi short with article two slightly less than one and a half times as long as article three. Hypostome length 0.486 mm.; corona medium in size with very slight apical notch; dentition 3/3, with 7 teeth per file and numerous small denticles extending about three-fourths its length.

Legs: Moderate in length. Coxa I with two short equal spurs; coxae II–IV each with a single spur as wide as long. Tarsus I without ventral terminal spur but tarsi II–IV with a very tiny ventral terminal spur.

Female (Fig. 2, G–K)

Body: Three unengorged specimens, exclusive of capitulum, 2.50, 2.60 and 2.60 mm. long and 2.40, 2.45 and 2.30 mm. wide, respectively. Punctations on body surface each containing a short white hair. Dorsal foveae and marginal grooves absent. Scutum of fifteen specimens 1.25 to 1.50 mm. long, average 1.37 mm. and 1.70 to 2.00 mm. wide, average 1.73 mm.; dark brown and ornate. Ornamentation consisting of three irridescent markings, variable in size and in shape; one on each side of cervical grooves and one median near posterior margin of scutum Punctations large, deep and uniformly distributed. Cervical grooves short, deep and convex externally. Festoons on unengorged females similar in appearance and in number to those of male but less distinct in partially engorged specimens and only faintly evident in fully engorged. Ventral body surface punctate, with each punctation containing a short, thick, white hair. Genital opening at level of coxae II and III in unengorged females but closer to level of coxae II in engorged specimens. Anal opening slightly below level of spiracular plates. Spiracular plates slightly longer and wider than those of male but essentially similar in form.

Capitulum: Fifteen specimens, from tip of palpi to tip of cornuae, 0.845 to 0.936 mm., average 0.888 mm.; length of basis from 0.312 to 0.338 mm., average 0.325 mm.; width of basis from 0.546 to 0.624 mm., average 0.582 mm. Basis sub-rectangular with lateral margins slightly convex externally. Cornuae small. Distance between porose areas slightly less than

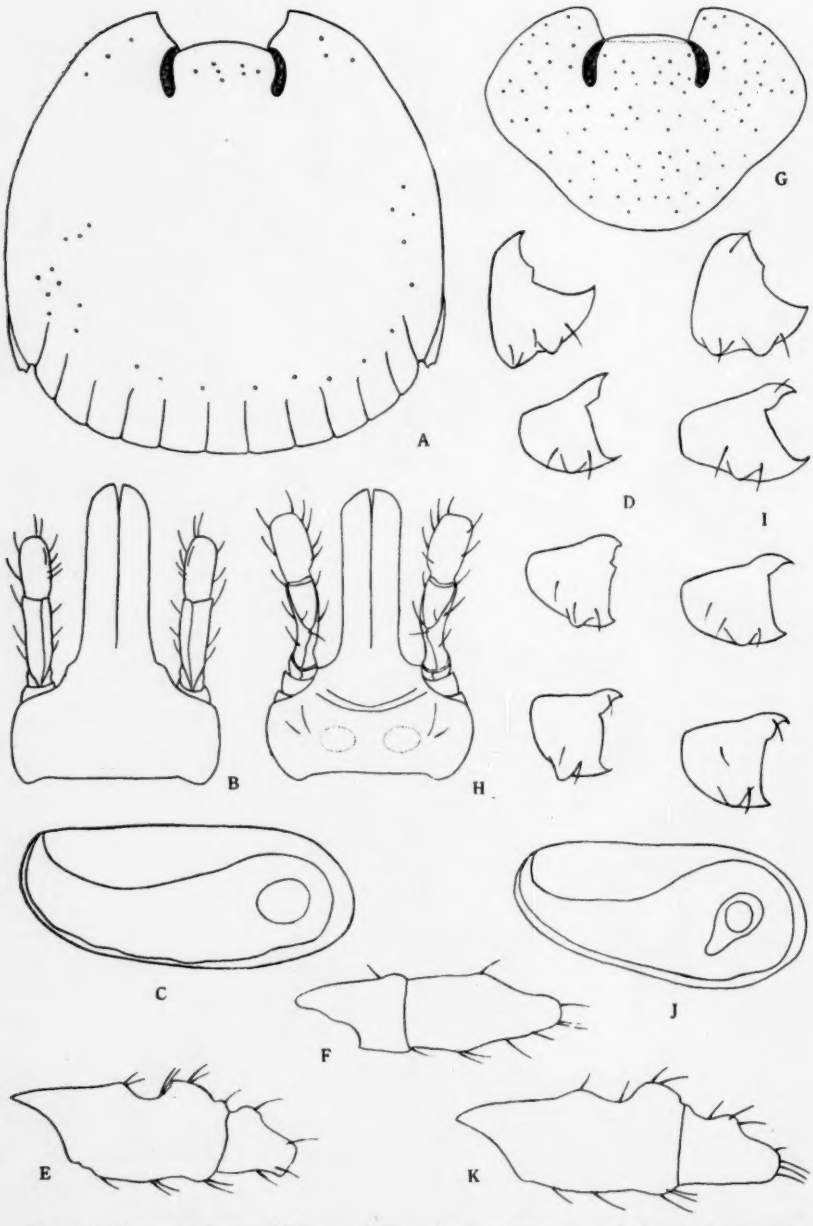

FIG. 1. *Aponomma kraneveldi* n. sp. A–F, male: A, scutum; B, capitulum in dorsal view; C, spiracle; D, coxae I–IV; E, Tarsus I; F, Tarsus IV. G–K, female: G, scutum; H, capitulum in dorsal view; I, coxae I–IV; J, spiracle; K, tarsus I.

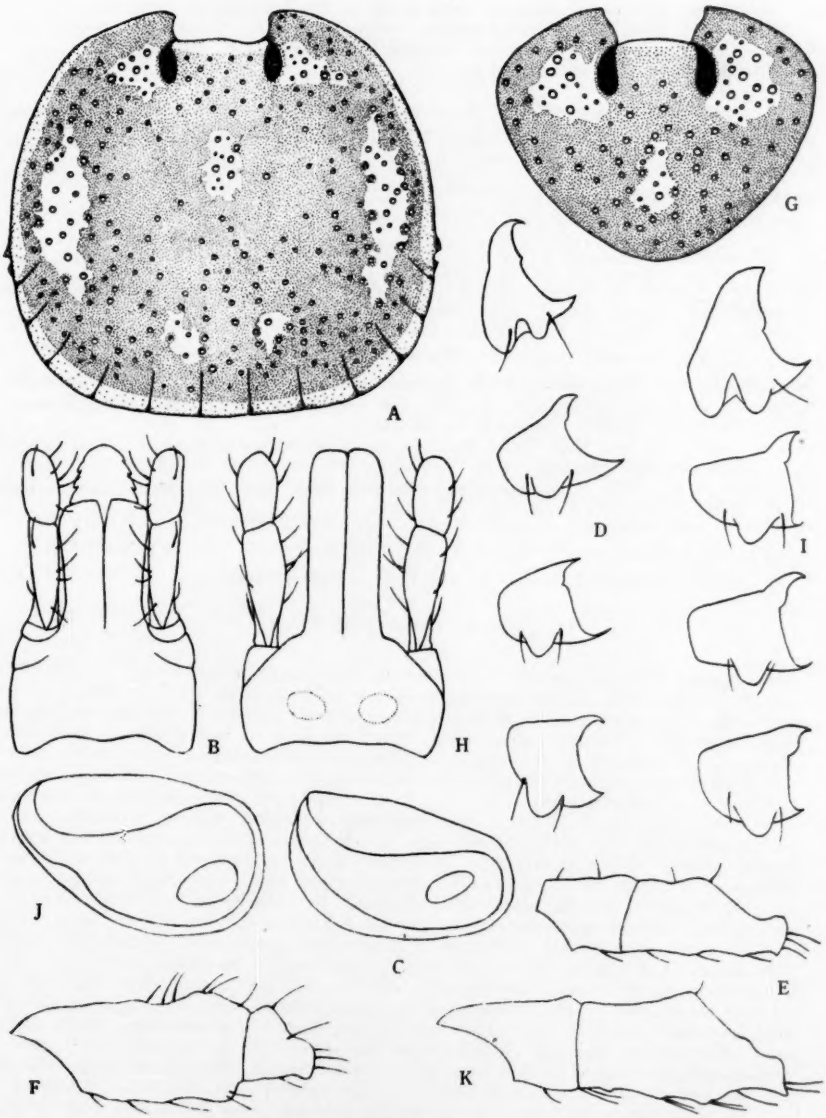

FIG. 2. *Aponomma soembawensis* n. sp. A–F, male: A, scutum; B, capitulum in dorsal view; C, spiracle; D, coxae I–IV; E, tarsus IV; F, tarsus, I. G–K, female: G, scutum; H, capitulum in dorsal view; I, coxae I–IV; J, spiracle; K, tarsus I.

their diameter. Palpi short with article two about one and a half times as long as article three. Hypostome length 0.520 mm.; in other respects similar to that of male.

Legs: Legs, coxae, coxal spurs and tarsi similar to those of male.

Holotype: Female, from *Varanus salvator,* Soembawa District, Soembawa Island, August 8, 1948. Deposited in the U. S. National Museum, Washington, D. C., no. 2221.

Allotype: Male, same data as holotype.

A. soembawensis resembled *A. komodoense* Oudemans, 1929, known only from Komodo Island, but it could be readily separated from it by the differences in the spiracular plates in both sexes. In addition *A. soembawensis* could readily be differentiated by its smaller size.

SUMMARY

Aponomma kraneveldi n. sp. and *A. soembawensis* n. sp. were described from Soembawa Island, Indonesia. Hosts were *Varanus salvator* (Laurenti) and snakes (determination unavailable), respectively.

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ISCHYROPODA FURMANI N. SP., A NEW ECTOPARASITIC MITE FROM UTAH

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In reporting on a series of mites collected at the Dugway Ordnance Project, Tooele, Utah, the writer (1953) discussed occurrence of several specimens of an undetermined species of genus *Ischyropoda* Keegan, 1951 in the collection. At that time a decision as to whether these specimens represented a new species was deferred, pending attempts to obtain additional material for study. Although no further specimens from Tooele have been obtained, two female examples, which may be assigned to this species, were sent to the writer by Dr. D. P. Furman of the Department of Entomology and Parasitology, University of California, Berkeley. Examination of this material has convinced the writer that these specimens represent a new species, which is here described as *Ischyropoda furmani,* so named in honor of Dr. Furman, who has made many excellent contributions to the field of acarology.

Genus *Ischyropoda* was proposed to include those laelaptid mites of subfamily *Haemogamasinae* Oudemans, 1926 in which leg II of both sexes possesses spurs, or massive, often blunt setae; anal shield of the male is separate; and accessory setae are present on the ventral shield of the nymph. There are two previously described species, *I. spiniger* Keegan, 1951 and *I. armatus* Keegan, 1951.

Ischyropoda furmani n. sp.
Figures 1–12

Distinctive characteristics: Female: Dorsal shield with a truncate posterior margin, which may be slightly concave; setae on shield and on dorsal body surface distinctly stouter than ventral body setae; many setae of dorsal shield barbed, or with bifurcate tips. A small, oval shield, which is smaller than the anus, lies on the dorsal midline posterior to the dorsal shield proper. Presternal area more lightly sclerotized than sternal shield; its sutures possess teeth. Tritosternum with a barb on either side of its basal piece close to the point of bifurcation. On either side of the tritosternum, adjacent to coxae I, are three pores arranged in a triangular pattern. Due to its weakly chitinized corners, the sternal shield is almost square; each of its margins may be slightly concave; usual sternal setae about equal in size, and almost as long as shield; in each of five female specimens examined there have been four accessory sternal setae; these are slightly more than half as long as the usual setae; all sternal setae smooth; length-width ratio of shield almost 1 : 1. Endopodal setae located directly behind the posterior pair of usual sternal setae; they are not inserted on the endopodal shields. Genitoventral shields relatively long and narrow; about as wide as anal shield, and not more than two-thirds as wide as sternal shield; usual genitoventral setae slightly shorter and more slender than anterior usual sternal setae; from four to seven accessory setae present on the genitoventral shields of specimens examined; the most anterior of the accessory setae is closer to the posterior margin of the shield than to the usual genitoventral setae; these setae are about two-thirds as long as the usual genitoventral setae, and about equal in size with adjacent body setae. Anterior margin of anal shield rounded; anus slightly more than its length from anterior margin of shield; paired anal setae inserted at a level near anterior end of anus; unpaired seta slightly longer than paired setae; unpaired and paired setae about equidistant from anus; no accessory setae present; length-width ratio of shield slightly greater than 2 : 1. Metapodal shields small, irregular ovals. Peritreme extends to level above portion of coxa II. Length-width ratio of tarsus I 6 : 1. Tibia II wth two stout, ventral setae. Tarsus II with three stout, ventral setae, and two blunt, terminal, spur-like setae. Epistome fimbriated, although its

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exact outline was difficult to determine in specimens available for study. Fixed digit of chelicera with a slender pilus and a bifid tip; movable digit with two teeth. Length of idiosome of holotype female is 768 microns; its width, between coxae II and III, is 494 microns.

Male: General aspects of setation as in the female. Posterior margin of the dorsal shield broadly rounded, rather than truncate, in the two specimens collected. The genitoventral portion of the sternigenitoventral shield slightly wider than the sternal portion; number and distribution of accessory setae as in the female. Anal shield as in female. Metapodal shields relatively longer than in the female, their length-width ratio being at least 2:1. Tarsal setae more slender than in the female. Length of idiosome of a paratype male was 669 microns; its width between coxae II and III was 426 microns; this specimen was slightly distorted due to mounting.

Nymph: On the single protonymph available for study, the posterior margin of the dorsal shield was concave. The small, dorsal platelet found posterior to the dorsal shield in male and female is also present in this stage. General aspects of setation are as in the adults, although there is only one pair of accessory setae in the sternal region. The two blunt, spur-like setae on tarsus II are as in the adults. There are no accessory anal setae. Due to distortion produced in mounting, length-width measurements would have little meaning.

Types: (In the following collection number citations DUG represents Dugway Ordnance Project, and RML represents Rocky Mountain Laboratory of the National Microbiological Institute. These specimens were forwarded to the writer through the Rocky Mountain Laboratory). *Holotype:* A female taken from *Microdipodops pallidus* at Tooele, Utah, 25 April, 1952. DUG No. 1211, RML No. 30376.

Paratypes: a female from *Perognathus longimembris,* Tooele, Utah, 7 August, 1951, DUG No. 183, RML No. 29263; a female from *Dipodomys ordii,* Tooele, Utah, 15 August, 1951, DUG No. 339, RML No. 29301; a male from *Onychomys leucogaster,* Tooele, Utah, 28 September, 1951, DUG No. 752, RML No. 29606; a male from *Peromyscus maniculatus,* Tooele, Utah, DUG No. 186, RML No. 29265; a nymph from *Microdipodops megacephalus,* Tooele, Utah, 7 August, 1951, DUG No. 178, RML No. 29262; two females from *Onychomys leucogaster,* 8 mi. N. of Mexican Water, San Juan Co., Utah, 28 August, 1953, D. P. Furman.

All type material has been deposited in the United States National Museum.

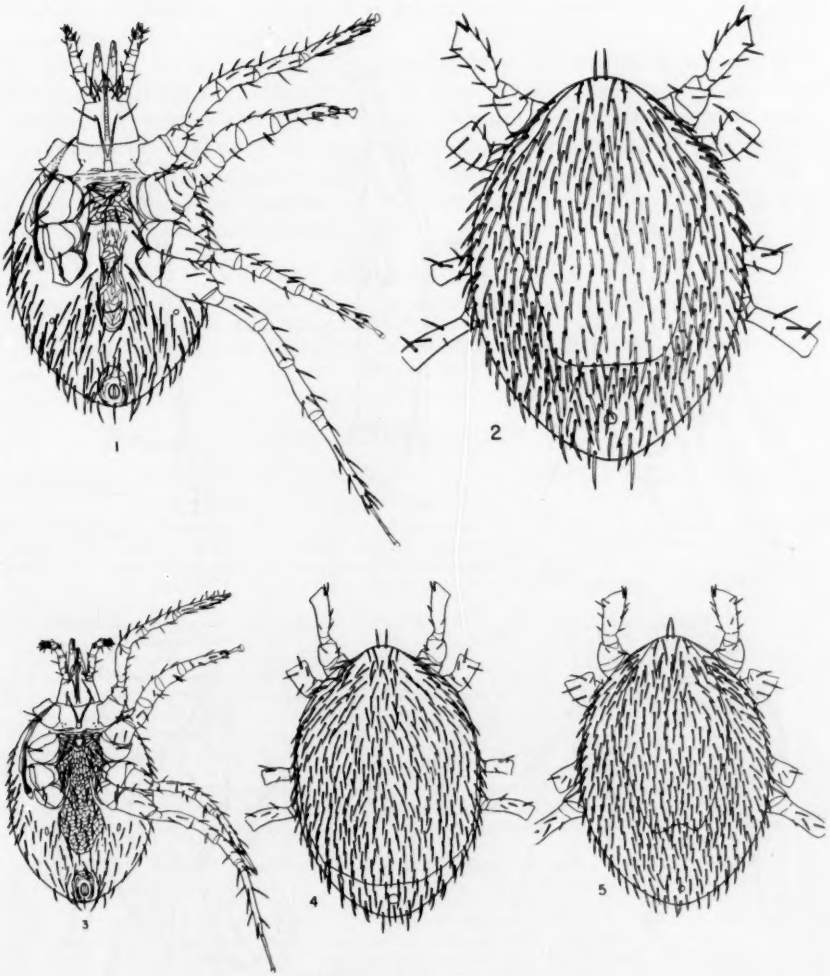
DISCUSSION

Ischyropoda furmani appears to be more closely related to *I. armatus* than to *I. spiniger,* the only remaining species of the genus. It may be easily distinguished from *armatus* in that female, male, and nymph possess two, rather than four, blunt, terminal, spur-like setae on tarsus II. There are numerous other differences in both setation and shield structure. Most conspicuous of these in the female is the genitoventral shield, which is about as wide as the anal shield in *furmani,* and over twice as wide as the anal shield in *armatus.* The male of *furmani* lacks the stout, ventral spur on the femur of leg II, which is present in *armatus,* and the ventral shield of the nymph of *furmani* possesses two, rather than six, accessory setae. An examination of a number of specimens of *I. armatus* has revealed that the small, oval platelet on the dorsal midline posterior to the dorsal shield is present in this species as well as *furmani.* This shield is similar to the small, posterior shields of *Allodermanyssus sanguineus* and *Ophionyssus natricis* and may indicate either that genus *Ischyropoda* should not be placed in subfamily *Haemogamasinae,* or that the existing concept of that subfamily should be changed. Possession of a single dorsal shield, in both adult and immature stages, has been regarded as one of the most distinctive characteristics of haemogamasid mites. Further study will be necessary before the true significance of this small, posterior shield can be evaluated. Its consistent location in all specimens examined is of particular interest.

SUMMARY

Ischyropoda furmani n. sp. is described as the third species of haemogamasid mite genus *Ischyropoda* Keegan, 1951. This species has been collected only at Tooele, Utah, and from an area 8 mi. north of Mexican Water, San Juan Co.,

PLATE I



EXPLANATION OF FIGURES

(All figures of *Ischyropoda furmani* n. sp.)*

PLATE I

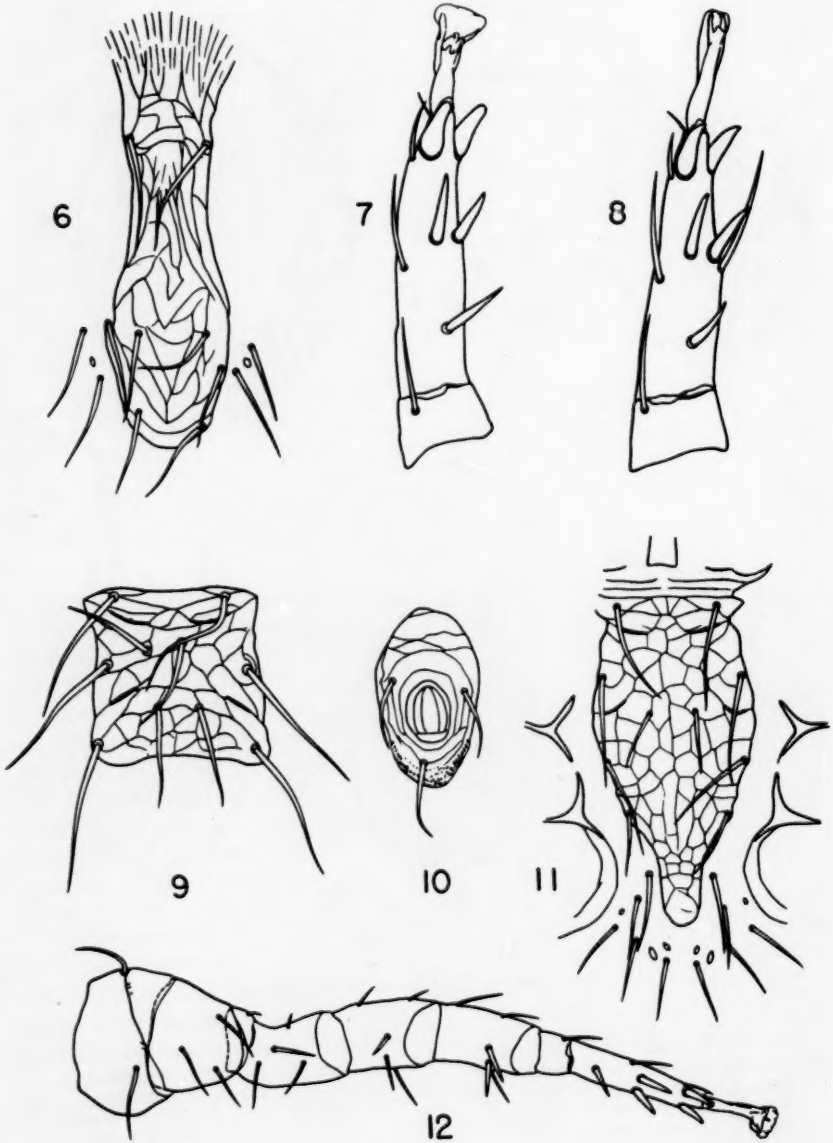
FIG. 1. Ventral view of female.
FIG. 2. Dorsal view of female.
FIG. 3. Ventral view of male.
FIG. 4. Dorsal view of male.
FIG. 5. Dorsal view of nymph.

PLATE II

FIG. 6. Genitoventral shield of female.
FIG. 7. Tarsus II of female.
FIG. 8. Tarsus II of nymph.
FIG. 9. Sternal shield of female.
FIG. 10. Anal shield of female.
FIG. 11. Ventral shield of nymph.
FIG. 12. Leg II of male.

* These figures were prepared by Mr. Saburo Shibata, Taxonomic Section, Department of Entomology, 406th Medical General Laboratory.

PLATE II



Utah. Recorded hosts have been *Dipodomys ordii, Microdipodops megacephalus, M. pallidus, Onychomys leucogaster, Perognathus longimembris,* and *Peromyscus maniculatus.* This new species appears to be most closely related to *I. armatus* Keegan, 1951. It may be easily distinguished from the latter in that tarsus II of all stages possesses two, rather than four, blunt, terminal setae. There are other differences in setation and shield structure. A small, oval shield or platelet found on the dorsal midline posterior to the dorsal shield is present in both *furmani* and *armatus;* this platelet is similar to that of *Allodermanyssus sanguineus* and *Ophionyssus natricis* and may indicate the unsuitability of the existing concept of subfamily *Haemogamasinae* Oudemans, 1926.

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RESEARCH NOTE

AMERICAN SHAD, *ALOSA SAPIDISSIMA,* PARASITIZED BY *ARGULUS CANADENSIS* IN THE CONNECTICUT RIVER

Ectoparasitic branchiurans were collected on June 17, 1955 from shad in the Connecticut River which were being passed over the Holyoke dam by means of a fish elevator. The branchiurans were identified as *Argulus canadensis* Wilson from Wilson (1944, Proc. U. S. Nat. Mus. **94** (3177): 568) by Dr. T. E. Bowman, Division of Marine Invertebrates, United States National Museum.

External parasites on shad from the Carolinas, the Woods Hole region, and the Connecticut River are mentioned by Yarrow (1874, Rept. U. S. Comm. Fish., 1872–1873, Part II: 456), Sumner, Osburn, and Cole (1913, Bull. U. S. Bur. Fish., 1911, **31,** Part II: 742), and Mansuetti and Kolb (1953, Chesapeake Biol. Lab., Pub. No. 97, Dec.: 19) respectively. The present report is the first one specifically listing *Alosa sapidissima* as a host for *Argulus canadensis.*

Argulus canadensis were first noted on some of the shad on June 11, nineteen days after the first fish in the spawning run had reached the Holyoke dam. They continued to be present on most of the 1668 shad passed between that date and June 28 when the run terminated.

On June 17 spawned out shad, called backrunners, began to enter the Holyoke canal system and congregate near the surface of the water in front of trash racks at industrial plants and power stations. Thirteen days later the first parasitized backrunner was captured. It is of interest that parasitized shad were not present among either the early upstream or downstream migrants.

When first observed on upstream migrants, the parasites were concentrated chiefly on the cheeks immediately ventral and posterior to the eyes. By June 20 they were common on the sides as well as the heads of the fish, particularly around lesions. The cause of these lesions was not determined, but similar ones were seen on unparasitized fish. Parasites were located on backrunners in positions similar to those on late upstream migrants.

Other fishes taken with shad in the fish elevator were not infected with *Argulus canadensis,* although this parasite has been reported from Lake of the Woods, Le Claire, Minn., on a species of whitefish, *Coregonus,* and on a rock sturgeon, *Acipenser fulvescens,* by Wilson (1916, Bull. U. S. Bur. Fish., 1914, **34:** 348). Wilson (1936, Canada Biol. Board Journ. **2:** 355) also reported it from Lake Ainslie and the Margaree River on Cape Breton Island, Nova Scotia, chiefly on brook trout although a few were found on stickle-backs, perch, and suckers. The United States National Museum has records of *Argulus canadensis* from *Coregonus sp.* Lake of the Woods, Canada and Minn.; *Coregonus clupeaformis* near Quebec City, Canada; *Acipenser fulvescens* Lake of the Woods, Canada; *Salvelinus fontinalis* Cape Breton Island, Nova Scotia, and Lake Kenogami, Quebec, Canada; *Gasterosteus wheatlandi* Cape Breton Island, Nova Scotia; and *Esox masquinongy* Trout Lake, Vilas Co., Wis.

Specimens of *Argulus canadensis* collected from *Alosa sapidissima* in the Connecticut River are in the United States National Museum with Catalogue Number 98464.—WILLIAM S. DAVIS, *U. S. Fishery Laboratory, Beaufort, North Carolina.*

NOTES ON THE GENUS *HEXOSTOMA* (MONOGENEA: HEXOSTOMATIDAE) WITH A REDESCRIPTION OF *H. EUTHYNNI* MESERVE, 1938

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Specimens of a monogenetic fluke belonging to the genus *Hexostoma* were collected by Dr. Clark P. Read from the gills of *Euthynnus lineatus* caught off the coast of Baja California between Abreojas and San Juanico, in 1952. The worms were referred to the writer for study and identification. Acknowledgment is made to Dr. C. P. Read for guidance during this study, to Dr. Boyd W. Walker for the collection of the fish, and to Dr. Emmett W. Price for the loan of type material from the U. S. N. M. Helminthological Collection.

Six specimens of the hexostomatid were available for study. Following fixation in Bouin's fluid, 5 of the specimens were stained with a dilute solution of either Mayer's haemalum or carmalum and mounted *in toto*. The remaining specimen was sectioned and stained with Harris' haematoxylin-eosin.

In the course of this study it was noted that discrepancies existed between the descriptions of *H. euthynni* Meserve, 1938 and *H. macracanthum* Fujii, 1944 and their respective types. Careful study of the types of these species indicated that *H. macracanthum* is not distinct from *H. euthynni* and should be considered a synonym of the latter. In view of this fact, a redescription of *H. euthynni* is presented herein together with a key to the species of *Hexostoma*. My specimens from *Euthynnus lineatus* are referred to the species *H. euthynni* and in so far as I am aware constitute a new host record.

Hexostoma euthynni Meserve emend.

Description: (All measurements in millimeters.) Body 3.57–5.85 long by 0.74–0.95 wide. Opisthaptor with 4 pairs of suckers arranged in 2 longitudinal rows, 3 anterior-most pairs approximately co-equal 0.153–0.255 long by 0.221–0.403 wide, posterior-most pair slightly smaller, 0.156–0.238 long by 0.194–0.332 wide (Fig. 1). Each sucker reinforced with 3 longitudinal sclerites, middle sclerite X-shaped, elevated ventrally from basal piece, lateral and medial sclerites double-pronged (Fig. 4). Opisthaptor with 2 pairs of hooks, large pair 0.085–0.120 long, and small pair situated between larger pair 0.024–0.034 long. Oral suckers 0.027–0.056 long by 0.024–0.040 wide. Pharynx 0.044–0.068 long by 0.026–0.036 wide. Genital pore ventral, median. Testes post-ovarian, median, 32–40 in number. Ovary median, in form of inverted U. Seminal receptacle near posterior end of ovary, large, 0.21–0.28 long by 0.068–0.119 wide. Vaginal opening dorsal, immediately posterior to genital atrium, provided with pair of denticulated bodies. Vitellaria extending from level of genital pore to posterior third of body. Eggs 0.103–0.221 long by 0.044–0.105 wide; anterior filament 0.100–0.179 long; posterior filament 0.100–0.161 long. Excretory pores situated anterior to genital pore on dorsolateral margins of body.

DISCUSSION

The genus *Hexostoma* as presently constituted contains 9 species: *H. dissimile* (Yamaguti, 1937), *H. thynni* (Delaroche, 1811), *H. acutum* (Goto, 1894), *H. grossum* (Goto, 1894), *H. extensicaudum* (Dawes, 1940), *H. euthynni* Meserve, 1938, *H. macracanthum* Fujii, 1944, *H. thunninae* (Parona and Perugia, 1889),

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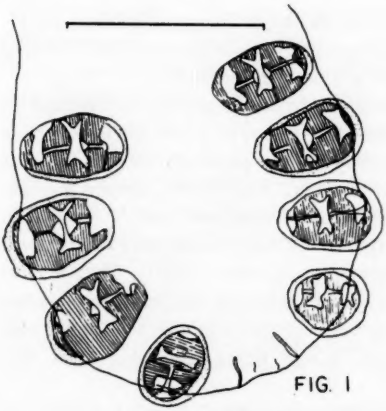

FIG. 1

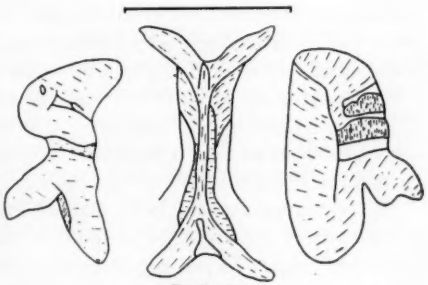

FIG. 2

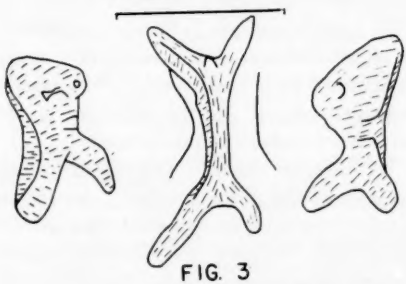

FIG. 3

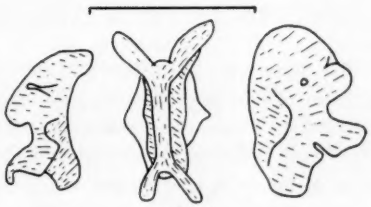

FIG. 4

EXPLANATION OF PLATE

All drawings made with the aid of a camera lucida.

Scale for Fig. 1, 0.5 mm.; for all others, 0.1 mm.

Figs. 2, 3, 4 medial sclerite on left, lateral sclerite on right.

FIG. 1. *H. euthynni* from *Euthynnus lineatus*. Opisthaptor showing longitudinal arrangement of suckers. (Details of sclerites not accurate)

FIG. 2. *H. macracanthum*. Sclerites of the 2nd right haptoral sucker. (Drawn from type)

FIG. 3. *H. euthynni*. Sclerites of the 3rd left haptoral sucker. (Drawn from type)

FIG. 4. *H. euthynni* from *Euthynnus lineatus*. Sclerites of the 3rd right haptoral sucker.

and *H. pricei* Koratha, 1955. These species fall into two clearly defined morphological groups. The first four species have the suckers of the opisthaptor in almost the same transverse plane with their long axes set longitudinally. The remaining five species have the suckers arranged one behind the other in two longitudinal rows with their long axes set transversely. Although previous investigators apparently have not considered these differences to be significant, I believe that they may constitute the basis for a generic separation of the two groups and deserve attention in future investigations of the HEXOSTOMATIDAE.

We are concerned here only with that group in which the suckers are arranged longitudinally. In this group only *H. extensicaudum* has the fourth or posteriormost pair of suckers considerably smaller than the other three pairs. In the remaining four species, and in my specimens the fourth pair of suckers is equal to or nearly equal in size to those of the other three pairs (Fig. 1).

H. pricei described by Koratha (1955) from the gills of *Sarda sarda* from the Gulf of Mexico differs from the other members of this group in the morphology of

the haptoral sclerites and in the greater size of the large haptoral hooks. In *H. pricei* the lateral and medial sclerites of each haptoral sucker are straight, whereas in the other species of this group these pieces are double-pronged.

In studying my specimens it was also necessary to study the type specimens of *H. macracanthum* and *H. euthynni*. Unfortunately the type of *H. thunninae* was not available to me; so it was necessary to rely on the published descriptions of Parona and Perugia (1889, 1892), Parona (1919), and Goto (1899) for this species.

H. thunninae appears to be distinct from *H. macracanthum* and *H. euthynni*. It differs from these species as follows: Larger body size; both pairs of haptoral hooks are larger; larger egg size; and fewer testes. Goto (1899), Parona and Perugia (1889, 1892), and Parona (1919) failed to mention the number of testes in their descriptions of *H. thunninae*. However, the illustrations of Parona and Perugia (1892) and Parona (1919) show 12 and 9 testes respectively for this species.

The description by Meserve (1938) of *H. euthynni* is cursory, and I agree with Sproston (1946) that "The description of the suckers appears to be oversimplified." In addition, Meserve described the cirrus of *H. euthynni* as consisting of a "cup-shaped body with two bean-shaped bodies attached to it posteriorly." My examination of the type reveals that the "bean-shaped bodies" are the armature of the vagina, a condition which also exists in all other members of this genus.

Meserve considered *H. euthynni* to be different from previously described forms on the basis of the morphology of the suckers and of the sclerites. According to Meserve the sucker is divided into 2 portions by a septum in which the middle sclerite is embedded. The "septum" present in *H. euthynni* was observed clearly by me in the type of *H. macracanthum*, in my specimens, and is depicted in the drawing of *H. thunninae* by Goto (1899). The "septum" appears to be part of the middle sclerite and is present in at least one other species of the genus, namely, *H. extensicaudam*. Dawes (1940) in his description of *H. extensicaudum* termed this structure a basal plate.

The other character employed by Meserve for distinguishing *H. euthynni* is the morphology of the sclerites. The three sclerites of each sucker according to Meserve are X-shaped. My examination of the type specimen reveals only the middle sclerite to be X-shaped, the lateral and medial sclerites on the contrary to be double-pronged (Fig. 3).

Fujii (1944) in his description of *H. macracanthum* states that this species most nearly resembles *H. euthynni* differing from the latter in four ways, namely, the structure of the sclerites, number of testes, length of the anterior egg filament, and the size of the posterior-most pair of suckers. Study of the type of *H. macracanthum* indicates that the morphology of the sclerites in this species is very similar to that found in *H. euthynni*. The lateral and medial pieces in both species are double-pronged (Figs. 2, 3). There is also no significant difference in the number of testes between these two species. Meserve gave the number of testes for *H. euthynni* as 26, but I was able to count at least 32 testes in the type specimen, a figure close to that for *H. macracanthum* which has 34–36 testes. Finally, examination of the eggs in the type specimen of *H. macracanthum* shows that the only anterior filament that can be measured precisely approximates in length the anterior filament of *H. euthynni*. Thus, *H. macracanthum* differs from *H. euthynni* essen-

tially in the size of the 4th pair of suckers. This difference is not as great as the measurements of Fujii indicate. My measurements of the suckers of the type specimens of both species are relatively close, and I do not feel the difference to be significant in view of the other similarities between these two species. For these reasons I believe that *H. macracanthum* is a synonym of *H. euthynni.*

My specimens are referred to the species *H. euthynni.* They differ from this species only in the smaller size of the suckers, and I feel that this difference is not sufficient to warrant creation of a new species for these forms.

The species of *Hexostoma* may be differentiated as follows:

Key to the species of HEXOSTOMA

1. a. All suckers of opisthaptor in two groups in one transverse plane 2
   b. All suckers of opisthaptor in 2 longitudinal planes 5
2. a. On left side of body 1st, 2nd, and 3rd suckers of increasing size, 4th one minute, on the right side only the large 3rd sucker present at side of the 4th minute one ... *H. dissimile*
   b. All suckers of opisthaptor developed on both sides of the body 3
3. a. Larger hooks of opisthaptor located in front of the smaller ones ..... *H. thynni*
   b. Large and small hooks of opisthaptor in almost the same transverse plane 4
4. a. Outer pair of hooks of the opisthaptor solid, about 0.090 mm. long ..... *H. acutum*
   b. Outer pair of hooks of the opisthaptor hollow, about 0.126 mm. long ..... *H. grossum*
5. a. Most posterior pair of suckers of the opisthaptor approximately one-half the size of the three more anterior pairs ..... *H. extensicaudum*
   b. Most posterior pair of suckers of the opisthaptor almost as large as the three more anterior pairs 6
6. a. Lateral and medial sclerites of haptoral suckers straight; large hooks of opisthaptor 0.145 mm. long ..... *H. pricei*
   b. Lateral and medial sclerites of haptoral suckers double-pronged; large hooks of opisthaptor less than 0.145 mm. long 7
7. a. Large hooks of opisthaptor 0.128–0.135 mm. long, short hooks 0.045–0.048 mm. long; twelve testes or less ..... *H. thunninae*
   b. Large hooks of opisthaptor 0.085–0.120 mm. long, short hooks 0.024–0.034 mm. long; more than twelve testes ..... *H. euthynni*

SUMMARY

Hexostoma euthynni is reported from *Euthynnus lineatus* which constitutes a new host record.

The genus *Hexostoma* is reviewed and *H. macracanthum* Fujii, 1944 is reduced to synonymy with *H. euthynni* Meserve, 1938. The emended description of *H. euthynni* is given.

A key is presented by which the eight known species of *Hexostoma* may be differentiated.

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TRICHOBILHARZIA BRANTAE N. SP. (TREMATODA: SCHISTOSOMATIDAE) FROM THE CANADA GOOSE (*BRANTA CANADENSIS* L.)

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In the course of an investigation of the cause of sickness and death among Canada geese in their overwintering grounds, carried on jointly by the Parasite Research Laboratory, Animal Disease and Parasite Research Branch and the Disease Laboratory, U. S. Fish and Wildlife Service, a blood fluke belonging to the genus *Trichobilharzia* was discovered. The species appears to be new, and for it the name *T. brantae* is proposed.

The long fragile flukes were difficult to remove intact, since they usually occurred in a network of veins. One specimen was removed from the mesenteric veins and the remaining specimens from the kidney veins. Occasionally a tangled knot of males and females was enmeshed within a vein in a gelatinous substance in which were also trapped numbers of eggs (Fig. A).

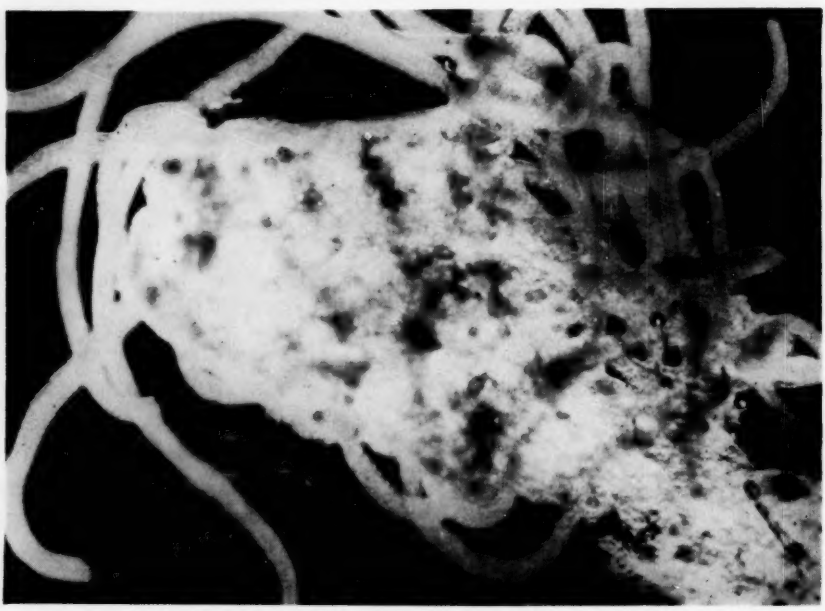

FIG. A. Flukes and eggs trapped in gelatinous material collected from renal vein.

The only other schistosome previously reported from the Canada goose is *Ornithobilharzia pricei* Wetzel, 1930, described from specimens collected from branches

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* Resigned July 17, 1953.

of the portal and mesenteric veins of a Canada goose captured at Back Bay, Virginia.

The following description is based on examination of two complete females, two complete males and fragments of both males and females.

Trichobilharzia brantae n. sp.

Filamentous worms as much as 50[1] or more long, somewhat flattened dorso-ventrally, with more or less parallel sides. Posterior end of body terminating in blunt fan-like structure, sometimes wider than any other part of body. Cuticle spinose. Spines small and blunt, sometimes lost, arranged in transverse rows. Oral sucker terminal, cone-shaped, with small spines on ventral surface of dorsal lip and lateral to mouth. Acetabulum larger than oral sucker, cup-shaped, retractable, covered with small spines. Esophagus long, with an expansion about midway between oral sucker and cecal bifurcation. Groups of longitudinal muscle fibers lateral to esophagus between expansion and cecal bifurcation, giving this portion of esophagus stiffened and thickened appearance. Esophageal gland cells surrounding muscle fibers and esophageal expansion. Esophageal bifurcation about two-thirds of distance between oral sucker and acetabulum. Cecal branches reuniting at different levels in males and females. Common cecum more or less spiral, extending into posterior expansion of body. Excretory pore not observed. Brain dorsal to esophagus just anterior to esophageal expansion.

Male.—Mature specimen, *holotype* (Figs. 3–6): Fragile, thread-like, 33.5[2] long. Body width, except for partially folded terminal expansion, varying from 0.063 to 0.180, widest in region of gynecophoric canal. Cuticle with few spines in scattered areas. Oral sucker 0.063 wide, 0.075 long, acetabulum, 0.082 in diameter. Anterior end of oral sucker 0.665 from acetabulum; acetabulum 0.280 from gynecophoric canal. Gynecophoric canal short and shallow, about 0.92 long, consisting of anterior, relatively non-muscular portion and arched, muscular, posterior portion. Posterior third of heavily muscular portion of gynecophoric canal lined with rows of stout spines. Genital papilla within gynecophoric canal near anterior margin of heavily muscular portion and at left of median line, armed with few relatively stout spines. Cecal branches reuniting just posterior to first loop of seminal vesicle, common cecum then following spiral course among testes to posterior end of body. Distance between anterior end of acetabulum and seminal vesicle 0.194. Seminal vesicle convoluted, sac-like, 0.425 long (ignoring convolutions), and as much as 0.053 wide, continuous with pars prostatica of similar appearance. Pars prostatica 0.221 long, constricted at posterior end into small bulb, opening into fairly straight ejaculatory duct terminating in muscular genital papilla. Both pars prostatica and ejaculatory duct surrounded by funnel-shaped thin-walled sac, probably a cirrus pouch. Testes numerous (totaling 585), beginning short distance behind gynecophoric canal and occurring in irregular groups on either side of common cecum to posterior end of body. Testes elliptical in lateral view, varying greatly in size, 0.017 to 0.05 in cross section. Dimensions of those in lateral view of mid portion of body (Fig. 5) 0.036 to 0.050 by 0.060 to 0.070. Testes connected by very short vasa efferentia to vas deferens, latter passing anteriorly and discharging ventrally into third loop of seminal vesicle (Fig. 3).

Female.—Mature specimen, *allotype* (Figs. 1–2): 18.5, fragment; fragile, thread-like, attenuated anteriorly, otherwise of more or less uniform width, 0.145 to 0.150. Oral sucker (in lateral view), 0.062 long and 0.055 deep; dorsal lip, about twice as thick as ventral lip. Acetabulum, about 0.08 in diameter. Cecal branches reuniting immediately caudad of seminal receptacle. Common cecum spiralling among vitelline follicles. Ovary convoluted, sac-like, 1.47 long (ignoring loops) and 0.022 to 0.044 wide, originating about 1.31 posterior to acetabulum. Seminal receptacle, sac-like, footprint-shaped, 0.223 long and 0.086 wide, connected to oviduct short distance behind ovary. Pore of Laurer's canal dorsal, immediately anterior to seminal receptacle, canal passing inward and forward to join oviduct. Oviduct passing posteriorly from ovary, uniting with anterior end of seminal receptacle, thence, oviduct turning forward in twisted loops joining Laurer's canal near caudal end of ovary, then passing below ovary and forward some distance cephalad of ovary to ootype. Ootype thin walled, leading into short muscular metraterm. Latter opening ventrally into genital pore just posterior to acetabulum. Vitelline follicles rounded or irregular, 0.009 to 0.034 wide, originating shortly caudad of seminal receptacle and irregularly scattered on each side of common cecum, posterior range not determined. Common vitelline duct extending forward ventral to seminal receptacle, paralleling ascending limb of oviduct, joining oviduct just caudad of ootype. Union of vitelline duct and

[1] Measurements in millimeters.

[2] A 22.5 mm fragment of anterior end and 11 mm fragment of posterior end are believed to be parts of the same individual.

oviduct not seen in this specimen. One smooth-walled, ovate egg with small recurved spine present in ootype.

Egg.—In ootype of mounted specimens, smooth-shelled, elongate ovoid, 0.025 to 0.035 by 0.052 to 0.061, bearing at broader end small recurved spine. Eggs from gelatinous substance from veins varying from 0.072 to 0.145 long by 0.044 to 0.089 wide. In freshly collected females, spine visible on egg while still in ootype, but only occasionally visible in mounted worms.

Host: Canada goose (*Branta canadensis*)

Location: Renal, iliac and smaller veins of kidneys, and mesenteric veins.

Locality: Pea Island and Mattamuskeet National Wildlife Refuges, North Carolina; Back Bay National Wildlife Refuge, Virginia; Blackwater National Wildlife Refuge, Maryland; Bombay Hook National Wildlife Refuge, Delaware; and Turnbull National Wildlife Refuge, Washington.

Specimens: U. S. National Museum Helminthological Collection No. 47607 (holotype), No. 47608 (allotype), and No. 47609 (paratypes).

The second entire male measures 42.28 long, 0.096 to 0.118 wide in the region of the testes and 0.191 at the posterior end. The oral sucker is 0.055 and the acetabulum 0.074 long. The distance from the anterior end to the acetabulum is 0.38, from the acetabulum to the gynecophoric canal, 0.63. Not all testes were observed, but 1490 were counted.

In fragments of males the oral sucker was 0.049 to 0.075 long by 0.055 to 0.075 wide; the acetabulum, 0.074 to 0.098 in diameter. The cecal branches reunite at slightly different levels in different specimens, ranging from anterior fifth to near posterior end of seminal vesicle. Vas deferens, when observable, unites with the seminal vesicle ventrally at about one-third to one-half the distance from the anterior end of the seminal vesicle. The less muscular portion of the gynecophoric canal varies in length, its folds arising anteriorly from the level of the anterior third of the seminal vesicle to the posterior third of the pars prostatica. The genital papilla is located near the anterior margin of the heavily muscular portion of the gynecophoric canal apparently either to the left or right of the median line. The number of testes apparently varies with the size of the fluke.

One entire poorly preserved female measured 41 long, 0.122 wide in the region of the ovary and 0.223 at the posterior end. Another entire female, also poorly preserved, measured 50 long. Other specimens were fragments of different lengths. In one fragment which showed the posterior expansion, the common cecum could be traced into the posterior end, but no vitelline follicles were found (Fig. 12). A detailed drawing of the ootype and metraterm (Fig. 10) made from one fragment and a drawing showing the posterior end of the ovary, anterior end of seminal receptacle and ducts uniting them (Fig. 11), made from another fragment, show relationships of the above-mentioned organs.

DISCUSSION

McMullen and Beaver (1945) included 11 species in the genus *Trichobilharzia* as follows: *T. burnetti** (Brackett, 1942), *T. corvi** (Yamaguti, 1942), *T. filiformis**† (Szidat, 1938), *T. horiconensis** (Brackett, 1942), *T. kegonensis** (Brackett, 1942), *T. kowalewskii** (Ejsmont, 1929), *T. ocellata**† (La Vallette, 1855), *T. physellae**† (Talbot, 1936), *T. stagnicolae**† (Talbot, 1936), *T. waubesensis**† (Brackett, 1942) and *T. yokogawai**† (Oiso, 1927). These authors believed that *Pseudobilharziella querquedulae* McLeod, 1937 was a synonym of *T. physellae* (Talbott, 1936) and that *Cercaria elvae* Miller, 1923 was a synonym of *T. ocellata* (La Vallette). More recently Neuhaus (1952) described *T. szidati**†

* Male described.

† Female described.

from the domestic duck and Wu (1953) described *T. cameroni**† from experimental hosts, ducks, canaries and pigeons. Wu was of the opinion that *Pseudobilharziella querquedulae* should be retained as a separate species, *Trichobilharzia querquedulae**† (McLeod, 1937). Macy and Moore (1953) briefly described *T. oregonensis** (Macfarlane and Macy, 1946). These authors raised the question as to whether *T. elvae* (Miller, 1923) Ameel, Van der Woude and Cort, 1953 was a good species or a synonym of *T. ocellata*. Macy, Moore and Price (1955) gave a more complete description of *T. oregonensis**† and at the same time indicated that the description of *T. ocellata* as presented by McMullen and Beaver (1945) should be applied to *T. elvae*. Edwards and Jansch (1955) described *T. adamsi*† from an experimentally infected duck. Fain (1955) described *T. anatina*,* *T. berghei*,* *T. nasicola*,*† *T. rodhaini*,*† *T. schoutedeni*,* and *T. spinulata**† from birds in the Belgian Congo. He did not compare his new species with any that had been described before.

The male of *T. brantae* can be differentiated from all other species except *T. stagnicolae*, the gynecophoric canal of which was not described, in that the gynecophoric canal is made up of a relatively non-muscular anterior portion and a muscular posterior portion and in that only the posterior third of the muscular part of the gynecophoric canal is lined with spines. In the other species the gynecophoric canal is described as consisting of a short muscular region, either completely lined with spines or without spines (that is no spines are mentioned in the descriptions).

T. brantae differs from *T. stagnicolae* in that the acetabulum is large and cup-shaped and the cecal reunion is at the level of the seminal vesicle while in *T. stagnicolae* the acetabulum is small and solid and the cecal branches reunite close to the acetabulum some distance anterior to the seminal vesicle; *T. brantae* differs from *T. berghei, T. corvi, T. filiformis, T. kowalewskii* (*Bilharzia polonica ex parte* as described by Kowalewski, 1896), *T. oregonensis, T. querquedulae, T. schoutedeni* in the level at which the cecal branches reunite; from *T. burnetti, T. cameroni, T. horiconensis, T. kegonensis, T. waubesensis* and *T. yokogawai* in that the vas deferens unites with the seminal vesicle caudad of the anterior end of the organ rather than terminally or posteriorly; from *T. ocellata, T. physellae* and *T. szidati* in that the oral sucker is smaller than the acetabulum; from *T. anatina, T. nasicola* and *T. rodhaini* in that the seminal vesicle is much longer than the pars prostatica; from *T. spinulata* in that the spines on the cuticle are short and blunt.

The elongate, ovate egg of *T. brantae* is similar to that of *T. filiformis*. It can, however, be readily distinguished from the crescent- or spindle-shaped eggs of *T. cameroni, T. elvae, T. nasicola, T. ocellata* (of McMullen and Beaver), *T. oregonensis, T. physellae, T. querquedulae, T. rodhaini, T. spinulata, T. stagnicolae, T. szidati, T. waubesensis* and *T. yokogawai*.

Only the posterior end of the female of *T. filiformis* was described by Szidat (1938). This portion differs from *T. brantae* in that the common cecum does not extend into the expanded posterior end of the body.

The presence of papillar spines near the anterior end of the female of *T. adamsi* differentiates this species from *T. brantae*.

The extreme length of the specimens described in this paper may not be a dis-

* Male described.
† Female described.

tinguishing character, since it is known that there is some variation in the length of the parasite which is dependent on the size of the host and the location within the host.

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Plate I

Explanation of figures.

Figures 1–7 and 12 drawn on scale given in Figure 1. Figures 8, 10–11 are drawn on scale given for Figure 10. Figure 9 drawn to scale indicated.

Fig. 1. Portion of anterior end of female. (type)

Fig. 2. Adjoining portion of same female.

Fig. 3. Anterior end of male. (type)

Figs. 4–6. Regions of testes of same male. Figure 4. Dorsal or ventral view. Figure 5. Lateral view. Figure 6. Posterior end.

Fig. 7. Posterior end of male.

Fig. 8. Egg containing miracidium.

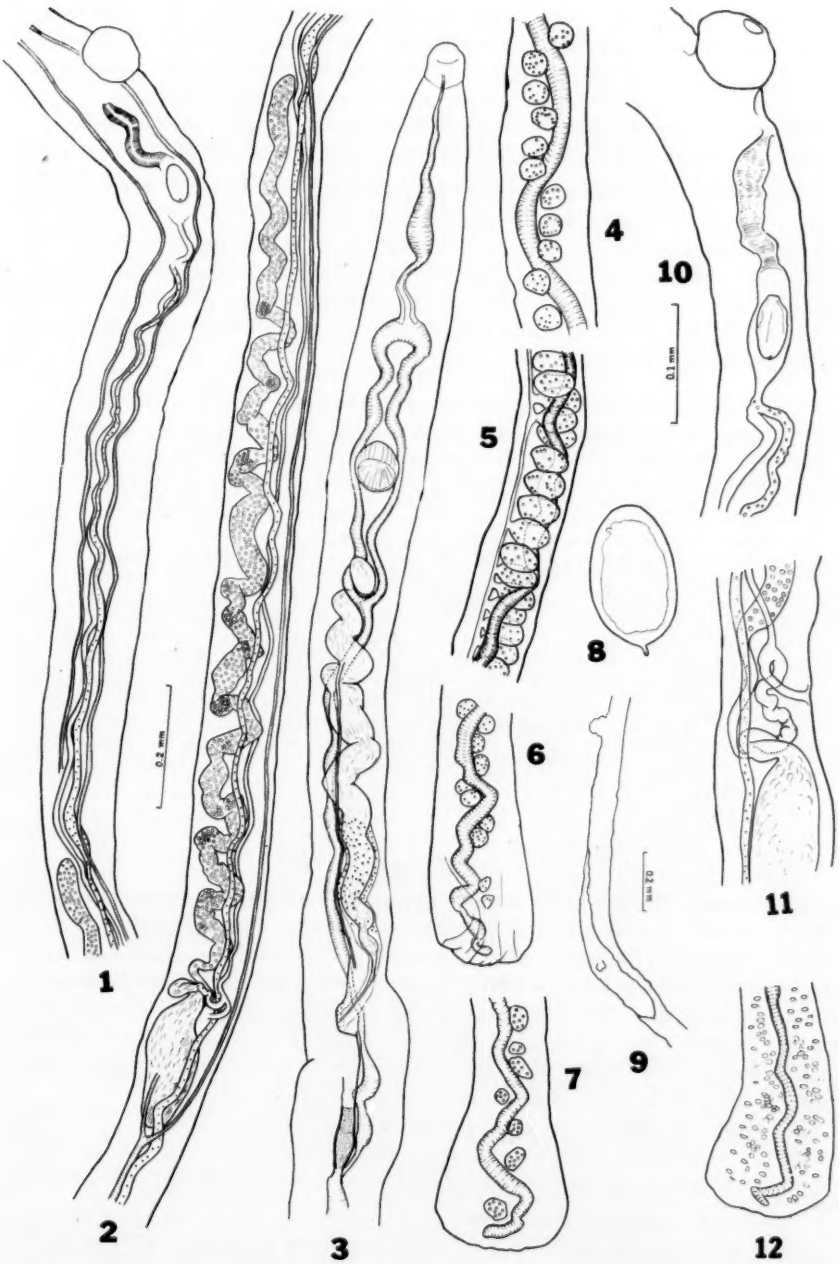
Fig. 9. Portion of male showing gynecophoric canal.

Fig. 10. Portion of female showing union of oviduct and vitelline duct, ootype containing egg and uterus.

Fig. 11. Portion of female showing posterior end of ovary, anterior end of seminal receptacle, oviduct, Laurer's canal and vitelline duct, lateral view.

Fig. 12. Posterior end of female.

PLATE I



TWO FLUKES, *PHAGICOLA MACROSTOMUS* N. SP. AND *PHAGICOLA BYRDI* N. SP., FROM THE TURKEY VULTURE (TREMATODA: HETEROPHYIDAE)

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Two small trematodes were recovered June 19, 1952, from the intestine of a turkey vulture, *Cathartes aura*. Both specimens proved to have characteristics of the heterophyid genus *Phagicola*, but neither one was referable to species so far described. Species diagnoses are given here, based on the specimens stained *in toto* after fixation under moderate pressure. The internal organization of both flukes was such that some structures and relationships not critical to species identification could not be fully studied.

Phagicola macrostomus n. sp. (Fig. 1)

Diagnosis. Small, spatulate worms about 0.5 mm long and half that broad. Body divided by a slight constriction at the level of the acetabulum. All but the posterior surface covered by semicircular cutaneous scales. Oral sucker diameter about one-fifth of body length, with no dorsal anterior prolongation. Acetabulum slightly more than one-half the diameter of oral sucker, center just anterior to middle of worm. Eighteen thick, blunt-pointed oral spines evenly spaced in a single row around oral opening, length about one-third of oral sucker diameter but somewhat variable. Oval diverticulum thick-walled, straight, overlapping pharynx for short distance. Prepharynx nearly as long as oral diverticulum. Pharynx muscular, twice as long as wide, divided by circular constriction. Esophagus shorter than pharynx. Ceca extending postero-laterally, then posteriorly to mid-ovarian level. Excretory pore posterior and terminal. Gonotyl indistinct, just anterior to acetabulum. Testes far posterior, nearly equal, zones nearly coincident. Ovary immediately anterior to and about size of right testis. Seminal receptacle spherical, medial to ovary, diameter about half that of ovary. Seminal vesicle large, U-shaped, with closed end of U to left of midline, posterior arm the larger. Vitellaria follicular, 8 to 12 per side, extending from near excretory bladder, ventral and lateral to testes, anterior to mid-ovarian level. Uterus extending lateral and ventral to ceca, one loop posterior to seminal receptacle. Undistorted uterine eggs moderately thin-shelled, amber, 14.5 to 15.5 microns long by 9 or 10 microns wide.

Host: *Cathartes aura* (turkey vulture)
Location: Intestine
Locality: Baker County, Georgia
Type specimen: U.S.N.M. Helm. Coll. No. 38115

Discussion: The previously described Western Hemisphere species of *Phagicola* are *P. minutus* (Looss, 1899), *P. angrensis* (Travassos, 1916), *P. nana* (Ransom, 1920), *P. longus* (Ransom, 1920), *P. diminutus* (Stunkard and Haviland, 1924), and *P. lageniformis* (Chandler, 1941). *P. macrostomus* differs from all these species in several respects. The oral sucker is larger; the oral spines either half as long or two to four times as long; and the anterior half of the body is much broader.

P. macrostomus differs also from *P. minutus* in having smaller eggs, longer ceca, larger gonads, and a larger acetabulum.

This species differs from *P. angrensis* in additional ways. More of its body is covered with scales; it lacks uterine loops anterior to the acetabulum; the acetabulum is more anterior in position; the seminal vesicle is anterior rather than posterior

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This work was done while the writer was an employee of the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Atlanta, Georgia, and Emory University Field Station, Newton, Georgia.

to the ovary, and is U-shaped rather than elongated; the vitellaria have a more extensive posterior distribution; and the eggs are smaller.

It differs from *P. nana* in that the oral spines are in a single rather than a double row dorsally; the acetabulum is more anterior; the eggs are smaller; and it lacks both an anterior prolongation of the oral sucker and a prominent gonotyl.

P. macrostomus is distinguishable from *P. longus* in other respects. The acetabulum is relatively larger; the bend in the U-shaped seminal vesicle is to the left rather than the right of the midline; the eggs, although smaller, are larger relative to the size of the worm than those of *P. longus;* and it lacks a bipartite gonotyl.

P. macrostomus is nearly twice as long as *P. diminutus;* it has cutaneous scales rather than spines; it has 2 more oral spines; the ceca extend posterior to the acetabulum, rather than ending at that level; the bend in the U-shaped seminal vesicle is to the left rather than the right of the midline, and the posterior rather than the anterior arm is the larger; the uterus is not arranged in transverse loops; and the eggs are considerably smaller than those of *P. diminutus.*

P. macrostomus lacks the well-developed dorsal anterior prolongation of the oral sucker found in *P. lageniformis;* it has a single rather than a double row of oral spines dorsally; it has an oral diverticulum which overlaps the pharynx, rather than falling short; and the ceca extend posterior to the acetabulum, rather than ending at that level.

Phagicola byrdi n. sp. (Fig. 2)

Diagnosis: Small, pyriform worms about 0.5 mm long and about two-fifths as wide at broadest part. Anterior half of body covered with semicircular cutaneous scales. Oral sucker diameter about one-tenth of body length, prominent dorsal anterior prolongation. Acetabulum oval, about three-fourths size of oral sucker, slightly posterior to middle of worm, its long axis forming an angle with the median longitudinal axis of the worm. Oral spines 16 in number, evenly spaced, points buried in soft tissue, length slightly more than one-third of oral sucker diameter but somewhat variable. Oral diverticulum long, thick-walled, overlapping pharynx then turning ventrally and anteriorly for a distance. Prepharynx length about twice diameter of oral sucker. Pharynx muscular, about one-half length of prepharynx. Esophagus short. Ceca extending posteriorly behind ovary, then medially for a short distance. Excretory pore posterior and terminal. Gonotyl prominent, immediately anterior to acetabulum, tri-lobed, with slit-like cavities, more than half its bulk to left of midline. Testes far posterior, at same level, equal. Ovary immediately anterior to right testis. Seminal receptacle medial to and considerably larger than ovary, overlapping it slightly. Seminal vesicle anterior to ovary. Vitellaria lateral, 9 or more follicles per side, extending from near excretory pore anteriorly into ovarian zone. Uterus in about 5 transverse loops between ovary and acetabulum, massed laterally. Undistorted uterine eggs moderately thin-shelled, amber, 18 to 19 microns long by 10 or 11 microns wide.

Host: *Cathartes aura* (turkey vulture)
Location: Intestine
Locality: Baker County, Georgia
Type specimen: U.S.N.M. Helm. Coll. No. 38116
This species is named for Professor Elon E. Byrd.

Discussion: Phagicola byrdi can be distinguished from *P. minutus, P. angrensis, P. nana, P. longus, P. dimunutus, P. lageniformis* and *P. macrostomus* by its extremely long, recurved oral diverticulum and the asymmetrically placed acetabulum. It can be distinguished from all but *P. nana* by its prominent, asymmetrically placed gonotyl.

P. byrdi differs also from *P. minutus* in having fewer and longer oral spines, longer ceca, larger gonads, and smaller eggs.

This species differs from *P. angrensis* in having very much shorter oral spines; perhaps in having a smaller oral sucker, although the size of that of *P. angrensis*

is given only as "large"; in having the acetabulum more anterior in the body; in lacking uterine coils anterior to the acetabulum; in the location of the seminal vesicle anterior rather than posterior to the ovary; and in a more extensive posterior distribution of the vitellaria.

It differs from *P. nana* in other details. Its cutaneous scales are broad rather than long; it has very much longer oral spines; there is a single rather than a double row of oral spines dorsally; the esophagus is shorter; the oral sucker is larger; the acetabulum is somewhat more anterior than in *P. nana;* and the vitellaria extend farther posteriorly.

P. byrdi is much smaller than *P. longus* but has slightly larger eggs; it has broad rather than long cutaneous scales; its oral spines are about the same size as those of *P. longus,* thus are larger relative to the size of the worm; the oral sucker is larger, relative to the worm's size; there are more vitelline lobules; and the gonotyl is trilobed rather than bipartite.

The ceca of *P. byrdi* are much longer than those of *P. diminutus;* its oral spines are longer; the worm is much longer than a large specimen of *P. diminutus;* and the vitellaria extend farther posteriorly.

P. byrdi is not as attenuated anteriorly as *P. lageniformis,* nor is the posterior half spherical; its intestinal ceca are much longer; it has 2 fewer oral spines, and the spines are in a single rather than a double row dorsally; its pharynx is somewhat larger; and the acetabulum is sunk in an obvious genital sinus, is asymmetrically placed, and is associated with a prominent gonotyl, none of which are characteristic of *P. lageniformis.*

P. byrdi differs from *P. macrostomus* in having a more attenuate anterior end, smaller oral sucker, smaller oral spines and fewer of them, and larger seminal receptacle.

Byrd and Reiber (1942) described a phagicolid from a muskrat as *P. nana.* Their specimen seemed to differ from the original description of that species in several details which the writer thinks are significant. Most outstanding is the small number of oral spines present in their specimen, it having 12 instead of the 16 or more in other species. It was noted that *P. lageniformis* has 18 spines, but no similar comparison was made with *P. nana,* which has 16 to 20. The specimen of Byrd and Reiber had a single row of spines dorsally, rather than a double row as described in *P. nana.* It also has a larger acetabulum situated more anteriorly than in *P. nana;* and an asymmetrically placed gonotyl, present in *P. nana,* was not described by Byrd and Reiber. The ceca may be shorter than in *P. nana,* but this could not be determined with certainty. It seems likely that the fluke from the muskrat might be a ninth species of the genus *Phagicola* in the Western Hemisphere.

It is regrettable that only one specimen of each of the new species described here was available, since the plasticity of trematode morphology makes a comparative study of abundant material highly desirable in defining new species. It also is probable that the vulture is an unusual host, and the effect of this on the morphology of the worms cannot be assessed. However, the combination of differences between these specimens and other described species seems greater than might be accounted for by individual variation or residence in an unusual host.

Phagicolids are found more usually in fish-eating animals. *P. minutus* has been reported from dog, cat and heron; *P. angrensis* was found by Travassos (1916)

in a heron; *P. nana* and *P. longus* were taken from an Alaskan fox in the Washington, D. C., Zoo by Ransom (1920), and it is not unlikely that the animal had been fed fish; *P. diminutus* was found by Stunkard and Haviland (1924) in rats from New York City which had ready access to marine fish; and *P. lageniformis* was taken by Chandler (1941) from a muskrat. It is probable that the host of the 2 species described here had eaten food not usually taken by vultures, and containing viable metacercariae. The bird then served as a satisfactory host in place of the normally fish-eating vertebrate such as a heron or muskrat.

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* The paper was published June 16, 1920, but is included in volume 57 of the Proceedings of the U. S. National Museum, dated 1921.

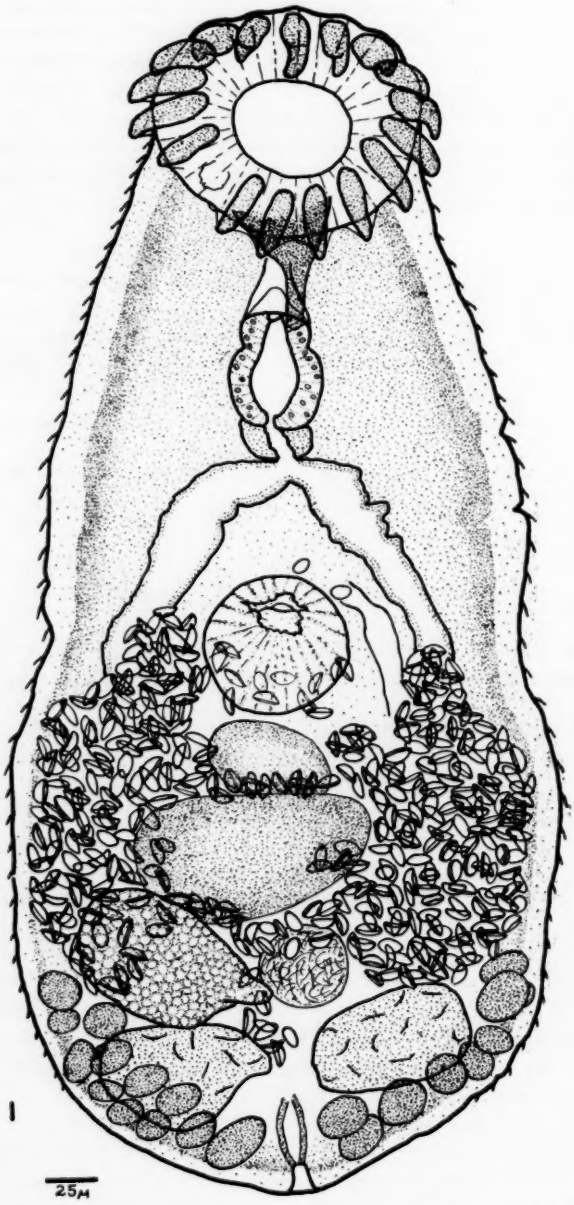

FIG. 1. *Phagicola macrostomus* n. sp. Ventral view, drawn with camera lucida. Ends of ceca not shown, one oral spine missing.

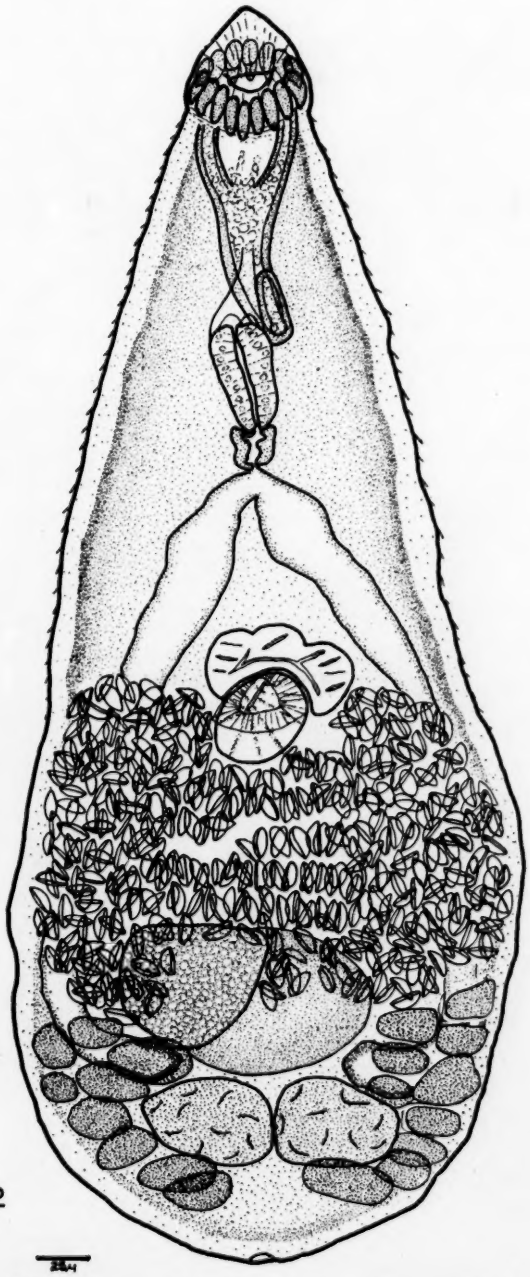

FIG. 2. *Phagicola byrdi* n. sp. Ventral view, drawn with camera lucida.

METAZOAN PARASITES OF THE BLUE JAY, *CYANOCITTA CRISTATA* L.

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Research dealing with avian parasites has been concerned primarily with those affecting domesticated birds. For the passerines, a study of the parasites of the eastern crow (Morgan and Waller, 1941) and one of the starling (Boyd, 1951) have been reported. The present investigation deals with the arthropods and helminths (in the coelom, gut and its derivatives) of the blue jay. Data from the files of the Helminthological Collection of the U. S. D. A. together with published records have been incorporated with the present findings so as to present a more complete survey of the parasites of this host.

MATERIAL AND METHODS

Between October 1947 and May 1954, 100 adult jays (both sexes in approximately equal numbers) were collected from New England: 1 from Vermont, 12 from New Hampshire, and the remainder from Massachusetts. All the months of the year are represented except August and September. A detailed table on the number of birds collected per month and their parasitic fauna is filed with the senior author. Each freshly killed bird was examined for parasites following the method described by Boyd (1951). Two additional procedures were introduced which revealed the presence of parasites previously overlooked. The first consisted of the eversion of the external ear, and the ticks and chiggers thus obtained are indicated by an asterisk in Tables I and IV. In the second technique the bird skin was finally shaken in 5% detergent solution, refrigerated overnight and the sediment examined. The resulting positive findings have been indicated in the Tables. The dissection of 6 individuals was omitted; so the percentage occurrence of helminths is based on 94 birds.

OBSERVATIONS

One of the 94 birds was unparasitized. The incidence was 83 for arthropods and 80 for helminths. No significant difference in the parasitic fauna was observed in the 2 sexes. With the exception of the liver flukes, infection per host was exceedingly light. The arthropods comprised 3 species of lice, 2 of ticks and 6 of mites (Table I). Sixteen species of worms were obtained: 4 flukes, 2 tapeworms and 10 roundworms (Table III).

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TABLE I.—*Arthropods recorded for the blue jay,* Cyanocitta cristata *L.*

Parasite	% incidence in present study	Published records	Localities
MALLOPHAGA			
Degeeriella eustigma	75	Peters, 1936	N.C., Va.
Menacanthus persignatus		Peters, 1933	Ala.
Philopterus cristata		Malcomson, 1929	Ill.
		Peters, 1936	Ala., Ga., Md., N.H., S.C., Va.
P. subflavescens	0	Geist, 1931	Ohio
Myrsidea funera	0	Peters, 1933, 1936	Ala., N.C.
Docophorus communis	0	Kohl, 1920	Pa.
DIPTERA			
Ornithoica vicina	0	Peters, 1933, 1936	Md., Mass.
		Bequaert, 1953	N.J.
O. fringillina	0	Bequaert, 1953	N.J.
SIPHONAPTERA			
Echidnophaga gallinacea	0	Trembley and Bishopp, 1940	S.C.
ACARINA			
Haemaphysalis leporis-palustris	1*	Bishopp & Hixson, 1936	Ga.
		Peters, 1936	Md., Va.
		Joyce & Eddy, 1943	Iowa
		Bequaert, 1946	Mass.
Amblyomma maculatum	0	Bishopp & Hixson, 1936	Ga.
Ixodes brunneus	0	Cooley & Kohls, 1945	Tenn.
I. scapularis	0	Cooley & Kohls, 1945	Ark.
Ixodes sp.	1*		
Ornithonyssus sylviarum	5†	Peters, 1936	Va.
Harpirhynchus sp.	2†		
Trombicula whartoni	11*	Brennan & Wharton, 1950	Kans.
T. alfreddugesi	0	Wolfenbarger, 1952	Kans.
Cytodites nudus	2		
Analgesidae		Robinson, 1955	Ga.
Analgopsis sp.	1		
Megninia aculeatus	0	Banks, 1915	N.S.
Proctophyllodidae		Robinson, 1955	Ga.
Proctophyllodes picae (?)	37		

* obtained following ear eversion in 58 birds.
† " " detergent technique used on 9 birds.

ARTHROPODS

INSECTA—MALLOPHAGA: Seventy-five blue jays harbored lice and/or their eggs. Infestation was extremely light; not a single bird was even moderately parasitized. Often it took a thorough search to find a louse, and sometimes eggs alone were collected. Immature stages were present consistently along with adults. The lice were identified as *Degeeriella eustigma* Kell. syn. *D. picturata* Osborn, *Philopterus cristata* Malcomson, and *Menacanthus persignatus* Kell. and Chap. The most prevalent, *D. eustigma,* occurred primarily on the breast feathers; *P. cristata* on the neck, and *M. persignatus* on feathers of the back.

Three kinds of louse eggs were identified (Figs. 1–9) and their characteristics have been summarized in Table II. Each kind was distinctive both with regard to the relation to its

TABLE II.—*Characteristics of the eggs of the three species of Mallophaga found on the blue jay*
(Egg measurements in microns)

Species	*Degeeriella eustigma*	*Philopterus cristata*	*Menacanthus persignatus*
Typical location	breast	neck	back
Type of feather	3.5 (2.5–5)	2 (2–2.2)	4.3 (2.5–5)
Feather length cm.	under surface barb	under surface barb	upper-lateral surface
Position on feather	1–8 mm from calamus	1–2 mm from calamus	proximal end rachis.
Attachment	basally by overlaping barbules	basally by conspicuous secretion	along its long axis by secretion
No. on feather	3 (1–11)	1 (1–2)	1
Egg			
Size ave. (mean)	655 by 200	830 by 380	955 by 372
Shape	'neck' at cap area square base	barrel conical base	lopsided distally oval base
Cap filament	present	absent	absent
Cap height	65	74	170
Micropyles			
Number	16–30	22–31	14, 15
Arrangement	irregular row	irregular row	regular row
Distance from edge	8	14	10
Dimensions	5 (rim 7.8)	6.6 (rim 9.7)	10 (rim 17)

feather attachment and the actual shape and size of the egg itself. Since nymphs of *D. eustigma* and *M. persignatus* were observed in the process of emergence from the shells of 2 of the egg types, the third type must represent the egg of *P. cristata*. The *D. eustigma* egg (Figs. 1–3), the smallest, is held to the under surface of the vane of the breast feather by the matting together of barbules, and is usually oriented somewhat obliquely with respect to the rachis. Often more than 1 is present on the same feather. This egg possesses a square base and distinct neck at the insertion of the cap. The cap bears a filament and numerous small micropyles, variable in number, arranged in an irregular row. The egg of *P. cristata* (Figs. 4–6) is located on the neck feather, usually 1 per feather. The attachment is also to the under surface of the vane but closer to the proximal end of the rachis than that of *D. eustigma*, and it consists of a conspicuous secretory material. The barrel-shaped egg has a conical base and lacks the distal filament. Its micropyles are similar to those of *Degeeriella* but are slightly larger. The egg of *M. persignatus* (Figs. 7–9), on the contrary, is located on the dorsal body feathers, typically 1 per feather. It is attached along its long axis to the edge of the upper surface of the proximal end of the rachis. The egg, the biggest of the 3 species, is somewhat lopsided with an oval base. The cap is large, lacks a filament, and its micropyles are also large, 14 or 15 in number, arranged in a regular row.

Louse-flies and fleas were not collected from any of these birds.

ARACHNIDA—ACARINA: Forty-nine blue jays were infested by acarines, 37 of them by feather-mites. On eversion of the external ears of the last 58 birds collected, ticks were found on 2 and chiggers on 11 jays (Tables I, IV). If the percentage incidence for ticks and chiggers is calculated on 58 instead of 100, then the 2% and 11% would become 3.4% and 19.0% respectively. Their presence was typically accompanied by vascular patches on the ear's surface and accumulations of waxy secretion. This condition was observed in 6 additional birds (5, Oct. 1951: 1, Feb. 1952) and might signify recent attacks by these parasites. The ticks were larval stages of *Haemaphysalis leporis-palustris* Packard and *Ixodes* sp., and occurred singly on hosts captured in Massachusetts (Oct. 1951: Oct. 1953). Mr. Glen M. Kohls, personal communication, states that it is impossible to identify the latter to species. The chiggers, identified as *Trombicula whartoni* Ewing, were collected from 4 New Hampshire birds (Oct. 1951) and 7 Massachusetts birds (3, Oct. 1951; 2, Oct. 1953; 2, Nov. 1951). The chiggers numbered 1 to 5 (average 2) per host, found in one or both ears.

One or two specimens of the blood-sucking mite, *Ornithonyssus sylviarum* Can. and Fanz. were taken from five birds (2, Feb. 1950; 1 each, May 1954, Oct. 1951, Nov. 1951). Its presence on one host and that of numerous mites, identified as *Harpirhynchus* sp., were revealed on two occasions when the detergent technique was used on the last 9 jays collected. *Cytodites nudus* was discovered twice in the trachea and bronchial tubes close to the syrinx. The one host (Jan. 1948) harbored 19 mites; the other (Oct. 1951) a single specimen. Feather-mites occurred in relatively small numbers per bird despite their high incidence of 37%. *Analgopsis* sp. was obtained only once (Oct. 1947) and from the under surface of the inner wing coverts. The rest, identified as *Proctophyllodes picae* (?), were mostly confined to the under surface of the flight feathers, particularly the secondaries, occasionally on neighboring body plumage. Eggs, immature and adult stages were present.

HELMINTHS

TREMATODA: Flukes were found in 2 locations, liver and cloaca (Table III). Liver fluke incidence was 30%; birds from all three states and from each month except July were involved. These flukes, comprising 3 species, ranged from 1 to over 100 blue jay, with an average of 20. Heavy infections resulted in inflammation of gall bladder and bile duct. All the hosts harbored *Lutztrema microstomum* Denton and Byrd; in addition one contained *L. monenteron* Price and McIntosh (Jan. 1952), and three *Zonorchis petiolatum* Denton and Byrd (May, 1951, 1954; Nov., 1951).

Five specimens of the cloacal fluke, *Leucochloridium cyanocittae* McIntosh were retrieved by the detergent method from one bird (May, 1954). An immature form was collected from each of the large intestines of two jays (Jan., Feb. 1950). Thus the percentage incidence is 3.2 for cloacal flukes.

CESTODA: Tapeworms were recovered only twice: a single specimen from a female (Oct., 1951) and three from a male (May, 1954). Unfortunately none of the scolices was retrieved thus making identification difficult.

Tapeworm from blue jay (Oct., 1951) (Fig. 10). Length approximately 150 mm. Proglottids short, wide, ranging from 70 by 500 microns to 300 by 1,100 microns. Genital pores irregularly alternate. Cirrus pouch distinct. Vas deferens coiled. Testes less than 10, between

TABLE III.—*Helminths recorded for the blue jay* Cyanocitta cristata *L.*

Parasite	Location in host	% incidence present study	Published reference or cat. No. Helm.Coll. U.S.D.A.	Remarks
TREMATODA				
Brachylecithum americanum	liver, bile ducts	0.0	Denton, 1945	Ga. imm. hosts
B. stunkardi	liver, bile ducts	0.0	Denton, & Byrd, 1951	Texas, Va.
Lutztrema microstomum	liver, bile ducts	29.8	Denton, & Byrd, 1951	Ga., N.C., Texas
L. monenteron	liver, bile ducts	1.1	———	
Lyperosomum oswaldi	liver, bile ducts	0.0	Denton, & Byrd, 1951	Texas
Zonorchis petiolatum	liver, bile ducts	3.2	Denton, & Byrd, 1951	Texas
Leucochloridium cyanocittae	cloaca	3.2†	McIntosh, 1932	Mich.
Collyriclum faba	skin of vent	0.0	Farner & Morgan, 1944	Md., Mich., Minn. Mass., N.J., Wisc. imm. hosts
CESTODA				
Oligorchis sp.	intestine	1.1	———	
Culcitella sp. ?	intestine	1.1	#39719	
ACANTHOCEPHALA				
Plagiorhynchus formosus	intestine		#29607, 1929	D.C.
NEMATODA				
Syngamus trachea	trachea	3.2	#33925, 1934	D.C. imm. host
Capillaria contorta	esophagus	20.2	———	
Dispharynx nasuta	proventriculus }	12.8	———	
Microtetrameres spiculata	proventriculus }		#27639; #31822, 1923, 1932	D.C., N.J.
			Boyd, 1956	Mass.
Acuaria anthuris	gizzard }	29.8	———	
Cheilospirura cyanocitta	gizzard }		Boyd, 1956	Mass.
Acuaria sp.	gizzard		Williams, 1929	
Spiroptera sp. (?)	?		#3850	
Capillaria obsignata	intestine	17.2	———	
Diplotriaena tricuspis	coelom	30.9		
Diplotriaena sp.	coelom		#2031, 1893	
"	coelom		#45290, 1946	Wisc.
"	coelom		#42395, 1934	Ont.
"	coelom		Robinson,* 1954	Ga.
Pseudoprocta sp.	wall of alim. tract	1.1	———	
Splendidofilaria sp. ?	wall of alim. tract	1.1	(Robinson, 1955 as unident. sp. same family)	Ga.

* Description of worms in 1954, identification given as *Diplotriaena sp.* in private communication.
† Obtained following detergent technique used on 9 birds.

excretory canals. Ovary and vitelline gland central. Uterus a transverse sac. Paruterine gland anterior to ovary and uterus.

The possession of the paruterine gland places this worm in PARUTERININAE, DILEPIDIDAE, but further identification is impossible without the scolex. However, in the Helminthological Collection of the U. S. D. A. is a cestode (#39719) collected from a blue jay in Washington, D. C. by M. F. Jones, June 1934. This was loaned to the senior author. Microscopic preparation shows a scolex containing 62 thorn-like hooks, arranged in two rows. The hooks (Fig. 11) of the anterior row measure 102 microns in length; while those of the posterior row are 65 microns long. Unfortunately, mature and gravid proglottids are lacking, but those present are somewhat similar to the specimen with irregularly alternate genital pores collected by us. If these two cestodes belong to the same species, then they cannot be in the genus *Anonchotaenia*, relatively common in passerines (Rausch and Morgan, 1947), since the scolex has hooks (Wardle and McLeod, 1952). They have been tentatively placed in the genus *Culcitella*, parasitic in hawks, on account of the occurrence of a transversely located uterus.

Tapeworm from blue jay (May, 1954). Four testes and unilaterally located genital pores identified this cestode as belonging to the genus *Oligorchis*, family HYMENOLOPIDIDAE. It bears a striking resemblance to *O. cyanocittii*, described from the stellar jay of Mexico by Coil (1955), in the position and size of the reproductive organs. Like the latter, the vagina enlarges proximally into a large conspicuous seminal receptacle, the proglottids are consistently broader than long, and a velum is present. Since scolex and gravid proglottids are lacking and it was collected from a different, though closely related, species of host, the 3 specimens of this tapeworm from the male blue jay have been tentatively identified as *Oligorchis* sp.

NEMATODA: Twenty-six of the dissected blue jays were negative for nematodes. Infection was scattered throughout the year. The greatest percentage infections, 30 and 32, are due to gizzard and filarial worms, followed by 20 and 17 due to esophageal and intestinal thread worms. The nematodes are described in sequence according to their habitation:—trachea first, then esophagus, proventriculus, gizzard, intestine and finally the body cavity (Table III). One pair of gapeworms, *Syngamus trachea* Montague, was present in the trachea of each of three jays,—one from New Hampshire (Oct., 1951) and two from Massachusetts (May, 1954; July, 1953). The average number of *Capillaria contorta* Creplin per host was 2.7 with a range of 1 to 25. However, of the 25 capillariids that occurred in the one bird (Feb., 1952), 16 were free in the mouth, indicating that they had not had time to become established in the jay.

Proventricular worms were present in 12 birds, and averaged 1.8 per individual, range from 1 to 5. Some were recovered from the scraped contents of the proventriculus; those partially embedded were usually surrounded by a mass of hypertrophied tissue in the form of finger-like projections of the wall. Two species were identified, *Dispharynx nasuta* Rudolphi and *Microtetrameres spiculata* Boyd. The former was more common and included 2 immature specimens. One of the immature specimens, a female, was 3 mm in body length, and its cordons, 300 microns for the posteriorly directed portion and 105 microns for the anteriorly directed portion. (Fully mature females measure 5.6–6.2 mm, and their cordons 525 and 190 microns.) The second immature specimen was 1.78 mm in body length with a corresponding cordon measurement of 137 and 34 microns. The species of the sexually dimorphic *Microtetrameres* was represented by 2 females and 3 males.

The gizzard worms averaged 1.8 per bird, range of 1–13. However, 23 of the 28 hosts harbored a single specimen, and the remaining five hosts contained 2 to 13 worms. Each worm was readily observed on the surface of the horny tunica facing the muscular wall by separating the tunica from the rest of the gizzard. On removal, a sinuous imprint of the nematode remained. The worms comprise 2 species. The first is the crow gizzard worm, *Acuaria anthuris* Rudolphi. It has long cordons and the males have similar spicules and 4 and 8 pre- and post-anal papillae. The more prevalent second species described by Boyd (1956) has now been named *Cheilospirura cyanocitta*. It has short cordons, with dissimilar spicules and 4 and 6 pre- and post-anal papillae in the males.

The intestinal threadworm, *Capillaria obsignata* Madsen occurred in both the small and large intestines, typically with the body partially embedded in the mucosa. The number of worms per bird varied from 1 to 9, mean 2.8.

The suborder FILARIOIDEA is represented by 3 species, the commonest being *Diplotriaena tricuspis* Fedtschenk, FILARIIDAE, with a percentage incidence of 31. It was consistently found in the body cavity, ordinarily adjacent to the gizzard. The average was 2.5 per bird, though 9 were found in 1 host. The other 2 species were retrieved from the gut wall (May, 1954; Oct., 1951). The filaria from the jay killed in May also belonged to the FILARIIDAE but to the APROCTINAE. Only a single specimen, a female, was obtained, partially embedded in the duodenum. This stoutish dark brown worm, without the male, has been tentatively classified as a species of *Pseudaprocta* Schikhobalowa.

Pseudaprocta sp. (Figs. 12–14) : Body length 17 mm. Width at head 150 microns; at base esophagus, 375 microns; near middle of body 600 microns. Mouth with four lips; tridents absent. Cuticular cordons delicate between cephalic papillae and amphids. Buccal capsule small. Esophagus simple, 660 microns long. Egg oval, thick shelled, 45–47 microns by 18–20 microns.

A jay examined in October harbored 13 filariids that have been identified as a species of *Splendidofilaria* Skrjabin, family DIPETALONEMATIDAE.

Splendidofilaria sp. (Figs. 15–17) : Anterior end lacking cephalic cuticular formation and cervical alae. Esophagus simple. *Male:* body length 6.84 mm. Width at base esophagus, 113 microns; at cloaca, 58 microns. Esophagus 410 microns. Tail 68 microns long. Spicules, right 68 and left 75 microns long. Pre-cloacal papillae 3. *Female:* body length 11.35–12.94 mm. Width, at anterior end, 123 microns; at base esophagus, 170 microns, at vulva level, 205 microns. Vulva 825 microns from anterior end.

DISCUSSION

Certain parasites previously overlooked in the routine examination of the bird's body were obtained by use of two procedures. Ticks and mites were discovered by everting the outer ears, and the *Harpirhynchus* mite and cloacal fluke by the detergent technique. This latter technique also accounted for a higher incidence of infestation by lice and blood-sucking mites.

A list of ectoparasites so far described from this host has been compiled from the results of the present study together with previously published data (Table I). The fluke that occupies cysts of the vent region, *Collyriclum faba* Kossack has been included with the endoparasites. Representatives, 1 each of 2 families of mites, constitute additional records for the parasitic fauna for this bird: *Harpirhynchus* sp., MYOBIDIIDAE and *Cytodites nudus,* CYTODITIDAE. The former is exceptionally small and appears to inhabit the surface of the skin of the host; the latter has been reported from the respiratory system including the air sacs and the body cavity of poultry (Baker and Wharton, 1952).

The data in the literature, except for the work of Robinson (1954, 1955), are the outcome of investigations that stressed the collection of parasites from a wide variety of animals, which happened to include the blue jay, rather than any concerted effort on this particular bird. Nevertheless, though our survey involved the examination of 100 jays, several ectoparasites already reported from this host were conspicuous by their absence. One possible reason for these negative findings is that of geographical distribution. Many of these parasites are limited to the southern part of the United States; others, for example some ticks, are more typical of the South where they exist in greater numbers. The flea, *Echidnophaga gallinacea* Westwood is a serious pest in the south but does not exist in Massachusetts, New Hampshire or Vermont (Trembley and Bishopp, 1940). The ticks, *Amblyomma maculatum* Koch and *Ixodes brunneus* Koch, *I. scapularis* Say are southern forms (Bishopp and Hixson, 1936; Cooley and Kohls, 1945) ; so also are the chiggers, *Trombicula whartoni* and *T.* (*Eutrombicula*) *alfreddugèsi* Oudemans, according to Brennan and Wharton (1950) and Wolfenbarger (1952). Collecting *T. whartoni* on birds from Massachusetts and New Hampshire is thus of special interest and constitutes two new state records for this chigger. The blue jay population in New England is composed of permanent and transient residents and probably the hosts for this chigger were migrant birds. Migrant animals often account for isolated records of southern species of parasites in the North.

The rabbit tick, *Haemaphysalis leporis-palustris,* has a widespread distribution and its immature stages have been reported several times from the blue jay. Joyce

and Eddy (1943) collected 4 nymphs and 30 larvae from 8 out of 12 jays in Iowa and they state that the tick occurs between April and September, the larvae being most abundant in August. Bishopp and Hixson (1936) took 4 nymphs and 10 larvae from a single bird in Georgia; they collected them from the head, especially the eyes and ears, but occasionally from other parts of the body. The only previous record from this host in Massachusetts is that of Bequaert (1946) who found a larva on October 15th. In the present study one larval rabbit tick and one of an unidentified *Ixodes* were obtained on October 20th and 11th, respectively. No birds, however, were examined in August and September, only 15 between May and July, and only 58 underwent ear eversion. Thus the percentage figures for ticks and chiggers on this host as given in Tables I and IV should undoubtedly be higher.

The survey comprised solely adult birds; juveniles were lacking. This is also important when one considers the association between bird and nest, parent and young relationships offer opportunity for the spread of parasites, and certain parasites tend to be nest-dwellers. These include fleas, hippoboscid flies and blood-sucking mites. Hippoboscids, according to Thompson (1937, 1938), may be collected between April and November, but especially in July and August. In this vicinity blue jays nest in May and June. Therefore a higher incidence of ectoparasites and the presence of the common hen flea and louse-flies would be expected in this survey had more birds, including juveniles, been collected in the summer months.

The egg of each of the 3 species of lice found on the blue jay shows distinguishing features. These characters are based on its position on the host, type of feather and attachment thereon, size and shape of egg and nature of its cap and micropyles. These observations substantiate those by Boyd and Chutter (1948) in their study of avian mallophagan eggs. Using the above bases of comparison they were able to identify the eggs of 10 species of lice examined by them.

The helminths reported from blue jays are listed in Table III. The data were obtained from our findings, previously published records and from the files of the Helminthological Collection of the U. S. D. A. Published material includes no cestodes or acanthocephalans and only a few nematodes.

Infection in blue jays by five species of liver flukes is reported by Denton (1945) and Denton and Byrd (1951) following an investigation of 700 birds from southern United States. New state records for two of these species are established in the present study:—Massachusetts, New Hampshire and Vermont for *Lutztrema microstomum,* and Massachusetts for *Zonorchis petiolatum.* The latter is of interest, since this species was taken previously from several birds including jays but only those occurring in Texas. The commonest liver fluke is *L. microstomum,* and according to Denton and Byrd (1951), it occurs in no other host and shows an infection rate of 27% with a range of 1 to 200 per bird, similar to the present findings. Of the other three species, *Brachylecithum stunkardi* Pande, they point out, is less common and again is found in no other bird. The blue jay is probably an accidental host for *B. americanum* Denton and *Lyperosomum oswaldi* Travassos, for only immature specimens of the former were obtained by Denton (1945) and from 1 of 12 birds; and the latter was reported by Denton and Byrd (1951) in 1 of 26 birds examined. The new and single occurrence of *Lutztrema monenteron* in

a jay (Jan., 1952) may be similarly accounted for. The cloacal fluke, *Leucochloridium cyanocittae* McIntosh was described from specimens in 2 of 4 adult jays dissected by McIntosh (1932). The 5 individuals obtained from a blue jay killed in May 1954 match this description except that the ovary touches or overlaps the posterior testis as it does in *L. actitis* McIntosh. For the fluke, *Collyriclum faba* Kossack, that inhabits cutaneous cysts in the vent region of birds, Farner and Morgan (1944) have presented a complete host list. They noted that it typically parasitizes young birds, so it is not surprising it was absent from the present survey.

The only acanthocephalan infection recorded for blue jays is the collection of 4 specimens by M. F. Jones in Washington, D. C., October 1929. They were tentatively identified as *Plagiorhynchus formosus* Van Cleave and catalogued in an unmounted condition as #29607 in the Helminthological Collection of the U. S. D. A. The material was loaned to the senior author, who on making permanent preparations verified the identification, since the nature of the hooks conforms with that described by Van Cleave (1942) for this species, reported from several avian hosts. He considered that it was confined to northeastern U. S. A. with Washington as its geographical center. However, it was later recorded from Alaska (Van Cleave and Williams, 1951).

The recovery of *Capillaria* from the esophagus and intestine and of *Dispharynx nasuta* from the proventriculus constitutes new records of nematode infection in the blue jay. Examination of freshly killed birds greatly aids their detection, which is almost impossible in preserved bodies. The previous record of gapeworms, *Syngamus trachea,* in blue jays was one from a nestling taken in June, 1934 by E. B. Cram and catalogued as #33925 in the Helminthological Collection. Goble and Kutz (1945a, b) and Madsen (1952) have shown that susceptibility to *D. nasuta* and *S. trachea* infections is greater in younger birds. Had juveniles been included in the present study it is likely that the percentage frequency for these two nematodes would have been higher than that obtained.

The two records of gizzard worm infections in addition to *Cheilospirura cyanocitta* described by Boyd (1956) consist of a *Spiroptera,* catalogued as #3850 in the Helminthological Collection, and an unidentified *Acuaria* obtained by Williams (1929) during a study of this genus. The *A. anthuris* in the present survey is similar to a new species, *A. nebraskensis,* which he collected from 8 of 9 crows, but which is regarded by Cram (1934a) as synonymous to *A. anthuris.* Material formally classified as *Spiroptera* now represents several genera according to Cram (1934a) including *Dispharynx* and *Cheilospirura,* so that the former record may even be a proventricular instead of a gizzard worm.

Filarial infections are strikingly high in the blue jay. Not only was there a 31% incidence of *Diplotriaena tricuspis* in the present investigation but there are 3 records of *Diplotriaena* sp. from this bird in the Helminthological Collection (#2031, 45290, 42395), 2 being from Wisconsin and Ontario. Robinson's studies (1954, 1955) on the filariae in Georgia bear this out. On examining 490 jays of varying age, he found microfilariae in 269 individuals, with a 65% incidence in adults (1955). In 1954 he observed at least 2 species of adult worms. One was stout, opaque, measured 2 to 17 cm long and was recovered from the coelom of 8 hosts. In a private communication to the senior author, he stated this nematode was a species of *Diplotriaena.* On further inspection, this species and the *Diplotriaena* speci-

mens in the Helminthological Collection will probably prove to be *D. tricuspis* since Robinson (1955) observed 1 of the microfilariae in the jay to be identical with that in the crow. The other mature worm was small, thread-like and inhabited the pericardial and peritoneal regions. According to Robinson (1955) both the microfilaria and adults possessed characters in common with the DIPETALONEMATIDAE. This worm may prove to be a species of *Splendidofilaria*. In the present study *Splendidofilaria* sp. was retrieved from the gut wall, and not under the skin nor in the heart region as is typical of the genus (Chabaud and Choquet, 1953). However, nematodes often exhibit a wanderlust, especially following death of the host. A species rather similar to this specimen was obtained from ground doves in the Virgin Islands by Augustine (1937). He described it as *Vagrifilaria columbigallinae* n. g., n. sp. According to Chabaud and Choquet (1953), this genus

TABLE IV.—*Comparison of percentage incidence of parasitism in the blue jay (present paper) with that in the crow (Morgan and Waller, 1941) and the starling (Boyd, 1951)*

	Blue jay adults	Crow adults	Starling juveniles and adults	
ARTHROPODS				
Mallophaga	75.0†	23.2	81.5	both
Mites, respiratory tract	2.0	–	4.3	"
Feather-mites	37.0	6.2	60.3	"
Skin mites	2.0†	–	5.9	adult
Ticks	2.0*	–	–	
Chiggers	11.0*	–	–	
Associated particularly with nest of bird				
Fleas	–	–	1.7	both
Hippoboscid flies	–	–	0.3	juv.
Blood-sucking mites	5.0†	–	3.8	juv.
HELMINTHS				
Trematodes	31.9	6.2	1.0	adult
Liver flukes	(29.8)	–		(0.3)
Intestinal flukes	–	–		(0.3)
Cloacal "	(3.2†)	–		(0.3)
Anal skin "	–	(6.2)		–
Cestodes	2.1 (2 spp.)	39.3 (2 spp.)	71.0 (3 spp.)	both
Nematodes				
Syngamus	3.2	– (exam. ?)	0.0	
Esophageal *Capillaria*	20.2	1.8	11.3	both
Proventricular worms	12.8	43.0	4.7	"
Gizzard worms	29.8	16.9	3.3	"
Intest. *Capillaria*	17.2	–	60.7	"
Diplotriaena tricuspis	30.9	5.3	–	
Misc. filarids	2.1	–	–	
Misc. intest. nematodes	–	–	5.7	"
Acanthocephala	–	–	6.0	"

* obtained following ear eversion in 58 birds.
† " " detergent technique used on 9 birds.

along with *Chandlerella* Yorke and Maplestone and *Parachandlerella* Caballero are synonyms of *Splendidofilaria*.

The only 2 extensive surveys in the United States on parasites of individual passerines prior to the present one are one dealing with the eastern crow (Morgan and Waller, 1941) and the other with the starling (Boyd, 1951). Since the blue jay belongs with the crow in the family CORVIDAE and all 3 possess characters in common, a comparison of their arthropods and helminths is presented in Table IV. The percentage figures are based on an examination of 100 adult blue jays, 112 adult crows and 300 juvenile and adult starlings. In general, the common name of the parasites is used, as the specific names ordinarily do not apply to all 3 hosts.

It should be noted as regards the lice, that the presence of their eggs alone was recorded as a positive finding in both blue jays and starlings, and this may account in part for their much higher incidence in these birds (75%, 82%) than in the

crow (23%). The absence of ticks and chiggers from the records for the crow and starling is not surprising since they were found only by everting the ear in the blue jay. Improved technique may alter the figures for the parasitic fauna of the crow, in particular with regard to the relative scarcity of ectoparasites and absence of intestinal *Capillaria*.

There was a marked contrast in degree of louse infestation per individual bird between the blue jay and starling. One had to search diligently on the former before finding lice, whereas the latter was typically "crawling" with them. Several factors are undoubtedly involved in the heavy louse infestation of the starling, in its high feather-mite incidence (60%) as well as in the occurrence of fleas and hippoboscid flies. In this investigation, 135 starlings were collected in August and September and approximately half of the 300 examined were juveniles. As already pointed out, a higher frequency of ectoparasites, especially of nest-dwellers, is to be expected in birds that have had recent association with the nest. Starlings, unlike jays and crows, nest successively in the same hole, having 2 or 3 broods per season. While investigating parasitism in nestling starlings the senior author (unpublished) was amazed to observe the increase in the parasitic population of the nesting site with the advancing weeks. The nest of the third brood was swarming with blood-sucking mites, which probably helped in causing the high mortality characteristic for these late nestlings over those hatched earlier.

The gregarious habit of the species is important as regards parasitism of the individual. Blue jays are relatively non-gregarious, compared with crows and starlings, which move in flocks and mass together in roosts at night. This fraternization affords opportunity for the spread of certain parasites. Another consideration involves the feeding habits of the bird. This applies especially to many endoparasites that require an intermediate host in order to fulfill their life history. The gapeworm and the 2 species of *Capillaria* are not in this category as they do not require a second host (Madsen, 1952). Blue jays are foliage seekers, three-quarters of their diet consists of vegetable matter (Forbush, 1927). The animal matter making up the remainder of their food is largely grasshoppers, caterpillars and fruit-eating beetles. It is not surprising that they show a high incidence of fluke infection (32%) as flukes have snails (vegetarians and dung-eaters) as their intermediate hosts. The incidence is in marked contrast to that of crows (6%) and of starlings (1%). Possibly there is a correlation between grasshopper consumption and the large number of jays infected by stomach worms (13%, 30%). Cram (1931, 1934a, b) demonstrated that the proventicular worm, *Microtetrameres helix* Cram, and the gizzard worm, *Acuaria anthuris*, of the crow, and species of *Cheilospirura* of poultry and game birds must pass through grasshoppers or crickets to complete their life cycles. Probably this holds true for the species of *Microtetrameres* and *Cheilospirura* of the blue jay along with the crow gizzard worm. Isopods act as the vectors for the other proventricular worm, *Dispharynx nasuta* according to Cram (1931) and for the acanthocephalan, *Plagiorhynchus formosus* according to Van Cleave (1942).

In the comparison of parasitism in the three hosts, one of the two most striking differences exhibited by the blue jay is its paucity of cestodes. Crows and starlings showed a 40% and 71% prevalence of tapeworm infection, respectively, whereas that in the jay was only 2.1%. It has been demonstrated that the immature stage

of the crow tapeworm, *Hymenolepis variabile* Mayhew is spent in the bodies of dung beetles and grasshoppers (Jones, 1934). Crows and starlings are omnivorous in their diet and tend to be ground-feeders and these facts may have a bearing on the frequency of cestodes in both.

The second major difference in the parasitic fauna of the three hosts lies in the high number of jays infected with filarial worms (31%). As a result of his study, Robinson has shown that the infection is contracted in the nest for he obtained microfilariae from many juvenile birds, from a jay approximately 5 weeks old (1954) and finally from a jay raised in captivity after having been removed from its nest when 16 days old (1955). He estimated that it took about 8 weeks for worms to reach maturity in blue jays. He observed that the microfilariae exhibit nocturnal periodicity. In discussing the possible vectors for these worms, Robinson suggests that gnats may act as the intermediate hosts. Engorged *Culicoides haematopotus* were found in the jay's nest and the gnats are to be found in abundance in the woodland nesting territory during the breeding season (Robinson, 1955). However, the possibility that the blood-sucking mites may serve as vectors should not be ruled out. These ectoparasites are known to occur in large numbers in the nest of starlings, yet they were present in only 3.8% of the 300 starlings investigated and all the hosts were juveniles. In contrast, 5% of the jays harbored them and these were all adult birds. It has been proved that species of *Bdellonyssus* may serve as vectors of St. Louis encephalitis virus and for the filarial worm of cotton rats (Baker and Wharton, 1952).

SUMMARY

1. One hundred blue jays were collected from New England (Mass., 87; N. H., 12; Vt., 1) between October 1947 and May 1954, all months being represented except August and September. The freshly killed birds were examined for arthropods and all but 6 individuals for helminths.

2. Arthropods were few in number on each host, and exhibited an 83% prevalence:—75% for 3 species of *Mallophaga;* 49% for 8 species of *Acarina* (37% for feathermites) (Table I). These figures should probably be higher since (a) ticks and chiggers were obtained only following eversion of the ears of the last 58 jays examined, and (b) the detergent technique for the last 9 individuals uncovered organisms that had previously escaped detection.

3. Original descriptions have been given for the eggs of *Degeeriella eustigma, Philopterus cristata* and *Menacanthus persignatus* (Table II).

4. Arthropods recorded for the blue jay from the present study and from published reports number 20 (Table I). The finding of *Harpirhynchus* and *Cytodites* mites constitutes new host records. New state records are established for *Trombicula whartoni.*

5. There was 80% infection of blue jays by helminths. Liver flukes (3 species) were found in 30%, a cloacal fluke in 3% and 2 species of cestodes in 2% of the birds. Nematodes included 10 species. Three percent of the jays harbored gapeworms; 20% and 17% esophageal and intestinal *Capillaria* respectively; 13% and 30% proventricular and gizzard worms (2 species of each) and 32% filarial worms (3 species, one of which, *Diplotriaena tricuspis,* was found in 31% of the birds) (Table III).

6. Twenty-one helminths are recorded for the blue jay from the present study

and from data in the literature and in the files of the Helminthological Collection, U. S. D. A. (Table III).

7. Six worms constitute new host records—*Lutztrema monenteron, Oligorchis* sp., *Dispharynx nasuta, Capillaria contorta, C. obsignata* and *Pseudaprocta* sp. To these may be added, *Acuaria anthuris, Diplotriaena tricuspis* and *Splendidofilaria* sp., where specimens belonging to these 3 families had been previously collected from this host but were unidentified as to species. Cestodes and acanthocephalans have not previously been reported for the blue jay but both are represented as unmounted specimens in the Helminthological Collection, each having been collected on 1 occasion. The single tapeworm (#39719) proved similar to 1 of the 2 recovered in the present study and identified as *Culcitella* sp.? The tentative identification of the acanthocephalan as *Plagiorhynchus formosus* has been confirmed.

8. The incidence of parasitism in the blue jay is discussed and compared with published findings in the crow and starling (Table IV).

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EXPLANATION OF FIGURES

FIGS. 1–9. Eggs of the three species of Mallophaga drawn in lateral view with the aid of a camera lucida.

FIGS. 1–3. *Degeeriella eustigma* Kell.; 1, under surface of breast feather with egg in situ; 2, cap area; 3, portion of edge of cap.

FIGS. 4–6. *Philopterus cristata* Malcomson; 1, under surface of neck feather with egg in situ; 2, cap area; 3, portion of edge of cap.

FIGS. 7–9. *Menacanthus persignatus* Kell. and Chap.; 1, upper surface of back feather with egg in situ; 2, cap area; 3, portion of edge of cap.

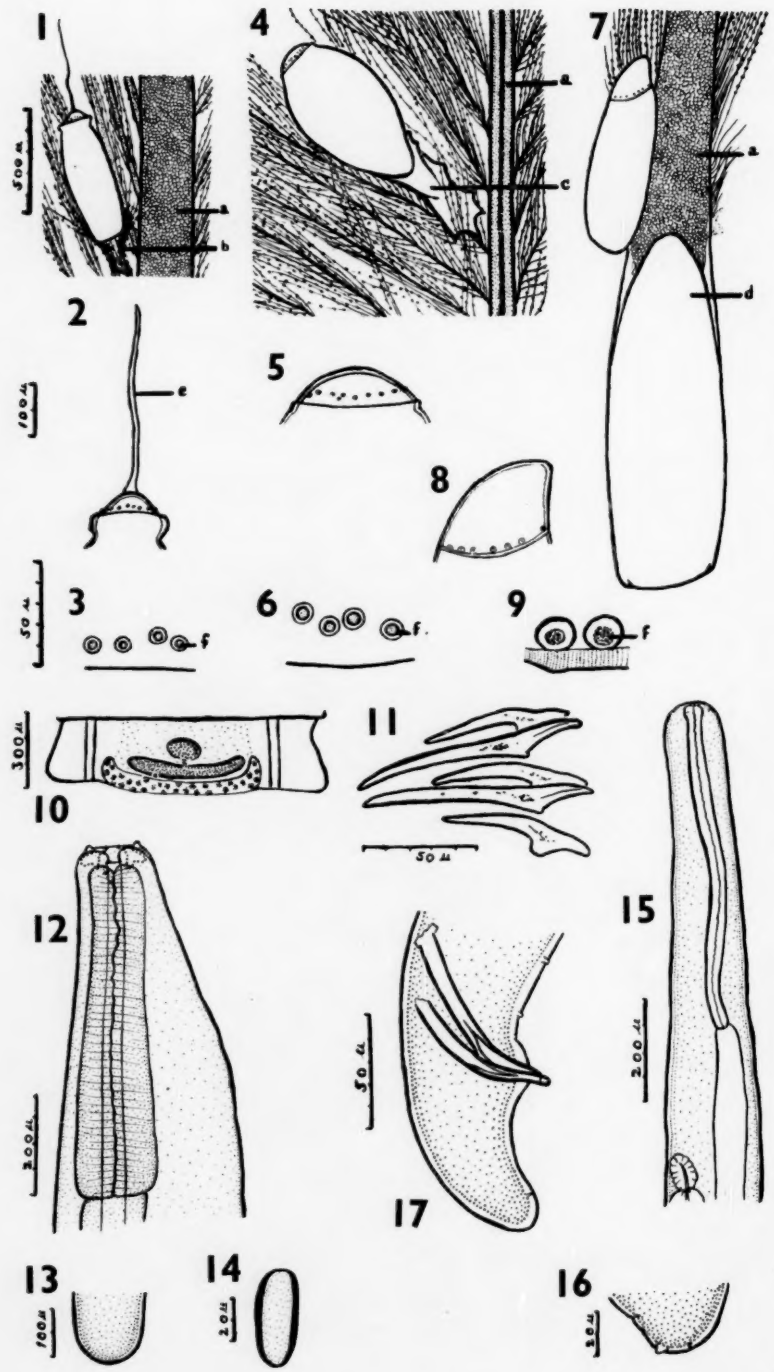
Abbreviations: a, rachis; b, barbules; c, secretory material; d, calamus; e, filament; f, micropyle.

FIGS. 10, 11. *Culcitella* sp. ? 10, mature proglottid of cestode from blue jay #63. 11, Hooks on scolex of cestode #39719 Helminthological Collection, U. S. D. A.

FIGS. 12–14. *Pseudaprocta* sp. Female. 12, anterior end. 13, posterior extremity. 14, egg.

FIGS. 15–17. *Splendidofilaria* sp. 15, anterior end female. 16, posterior tip female. 17, posterior region of male to show spicules and tail.

PLATE I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ERRATA

Page 138, vol. 42 (2) should read as follows:

tained nothing but the snails. In Experiments 9 and 18, the snails were maintained on sand.

All experiments were conducted at laboratory temperature which varied little. During the dry season the temperature ranged from 27 to 30° C., while during the remainder of the year the range was from 25 to 28° C. The relative humidity of the laboratory was more variable, but it usually ranged from 65 to 75 percent during the dry season and from 75 to 90 percent in the wet season.

In certain experiments, the jars were kept on the laboratory bench top, and the temperature and humidity conditions for these experiments were approximately those of the laboratory. In other experiments, the jars were set in pans of water which raised the relative humidity within the jars to 90–95 percent. In still other experiments, the jars were placed in large wooden cabinets where the humidity could be maintained at a relatively constant level and measured with a hygrometer.

In all experiments, the snails were examined periodically for viability by passing a strong beam of light through the shell in a darkened room. This was considered the best method for judging the viability of the snails without disturbing them or putting them in water. The accuracy of the method was tested by putting snails in water after they were examined in the light beam. The method proved to be reasonably accurate, but occasionally a dead snail was judged to be alive. In a living snail retracted into the shell, the part of the shell occupied by the snail body is opaque and the free boundary of the foot makes a sharp, straight line readily seen through the shell in transmitted light. In dead snails the free margin of the foot is not straight and sharp, and the part occupied by the snail body is not uniformly opaque because of shrinkage of the tissues away from the shell or the formation of gas and fluid within the body after death. As the snails dried they retracted farther and farther into the shell, and it became increasingly difficult to

Page 94, vol. 42 (1): In Table I, Compound incidence under "Peasant Children" should read "11" instead of "24."

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Diseases in Wild Mammals and Birds

Compiled by PATRICIA O'CONNOR HALLORAN, D.V.M., staff veterinarian, The Staten Island Zoo, Staten Island, N. Y.

Dr. Halloran assembled the bibliography during the past 10 years to provide reference material on the occurrence of diseases in wild mammals and birds in the free state or in captivity. The material is arranged in an orderly index to illustrate the incidence and character of disease patterns in the various animal groups. The references cited were obtained from more than 900 different publications throughout the world covering 120 years (1830–1950).

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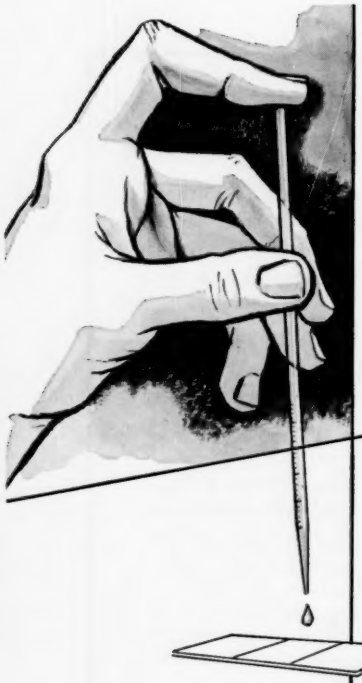

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